CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2016-2017 :
"Épigénétique et ADN égoïste"

27 Février, 2017

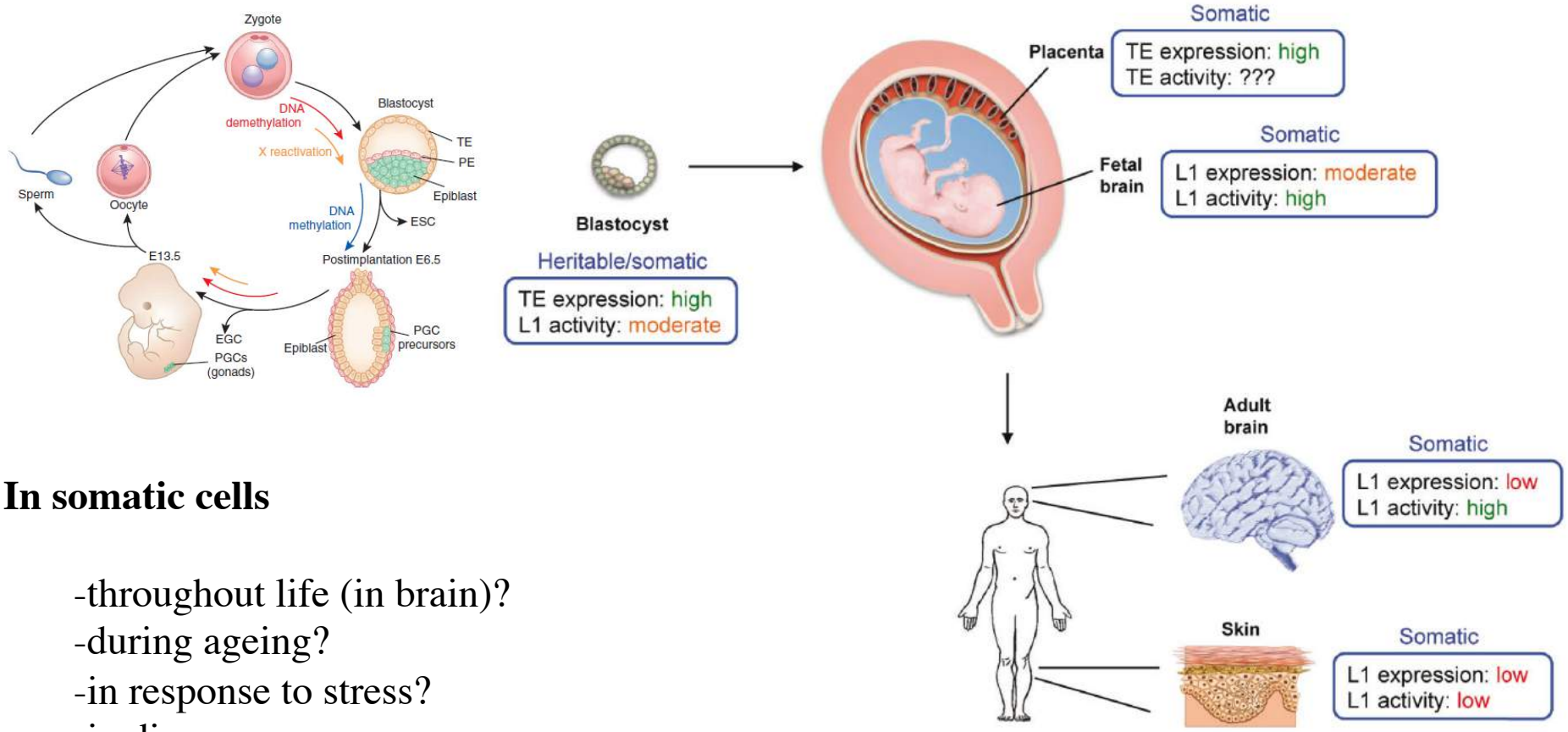
Cours IV

L'implication des éléments transposables dans les maladies : mutations et épimutations

The implication of transposable elements in disease: mutations and epimuations

TEs are Globally Silent but occasionally Active during Normal Life Cycle

During development and in the germ line

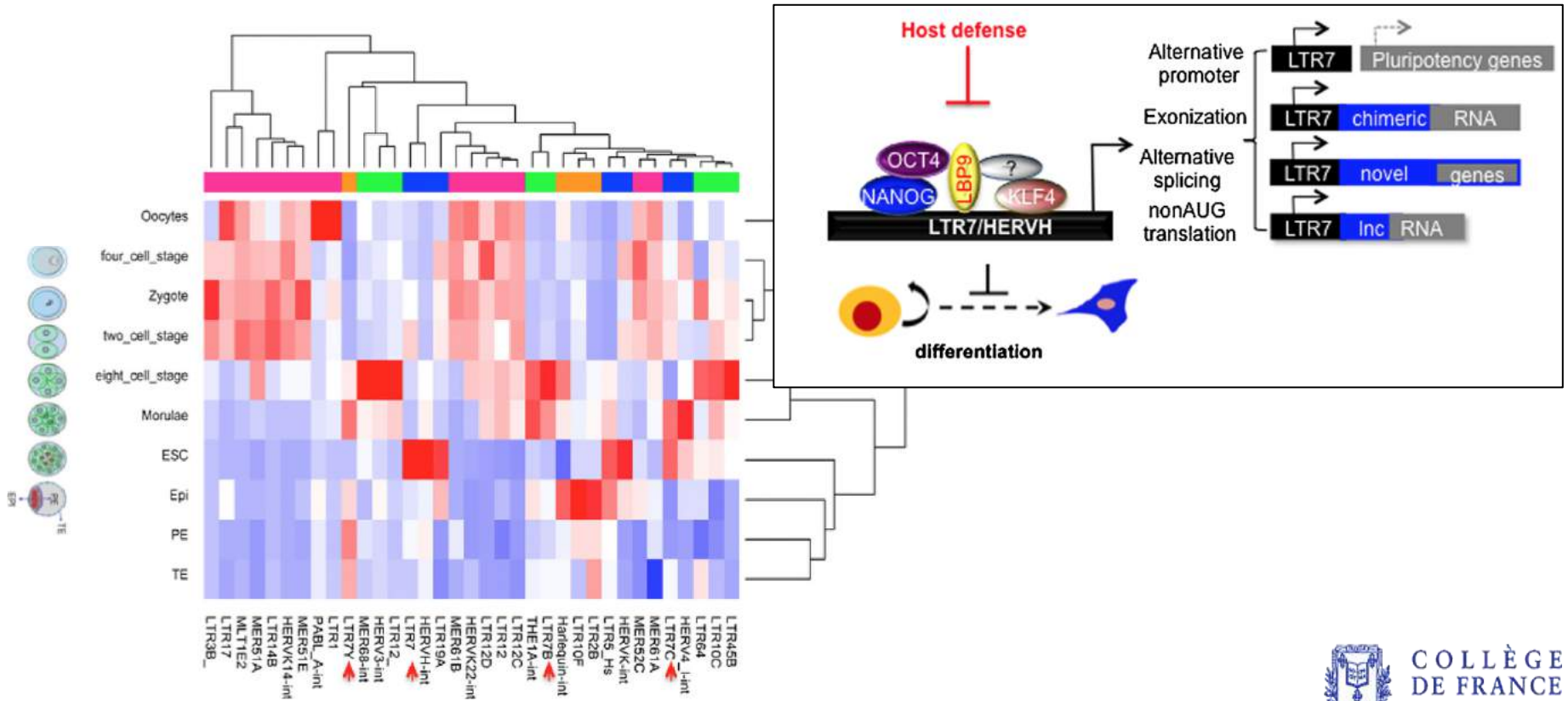


In somatic cells

-throughout life (in brain)?
-during ageing?
-in response to stress?
-in disease

TEs are Dynamically Expressed during Development

1. Distinct classes of TEs expressed in mouse and human pre-implantation development
2. Not *entire* subclass active at any given time but a specific subset of integrants – due to combined influence of trans-activators/repressors and local chromatin constraints – ***Cause or consequence*?**
3. TE-derived mRNA chimeric transcripts as well as long non-coding RNAs may play specific roles in development (eg in pluripotency in humans)



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4. Induction of HERV-K particles in early embryos may induce host viral restriction pathways to protect from subsequent infection by *exogenous* viruses?

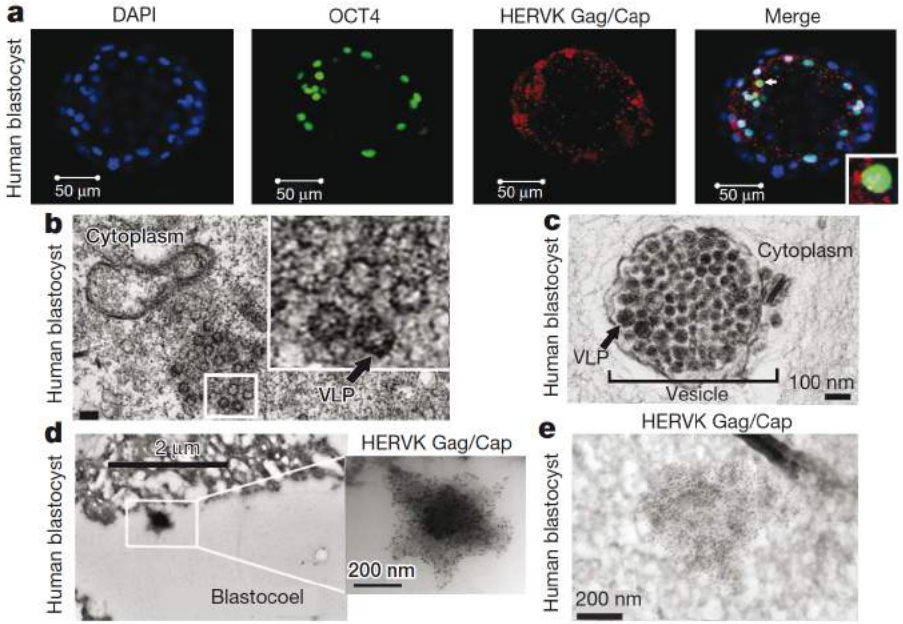
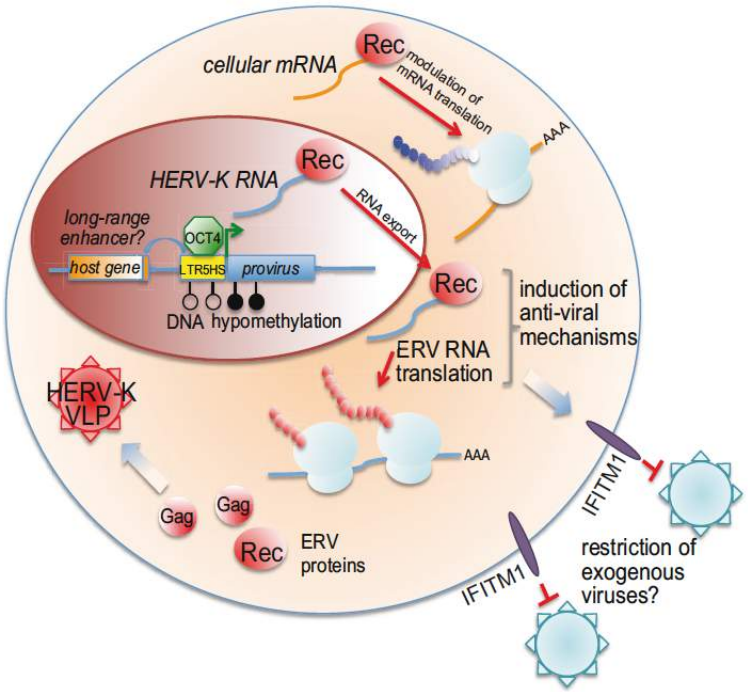


Figure 3 | Human blastocysts contain HERVK proteins and viral-like particles. a, Immunofluorescence of human blastocysts (days post-fertilization



E. H

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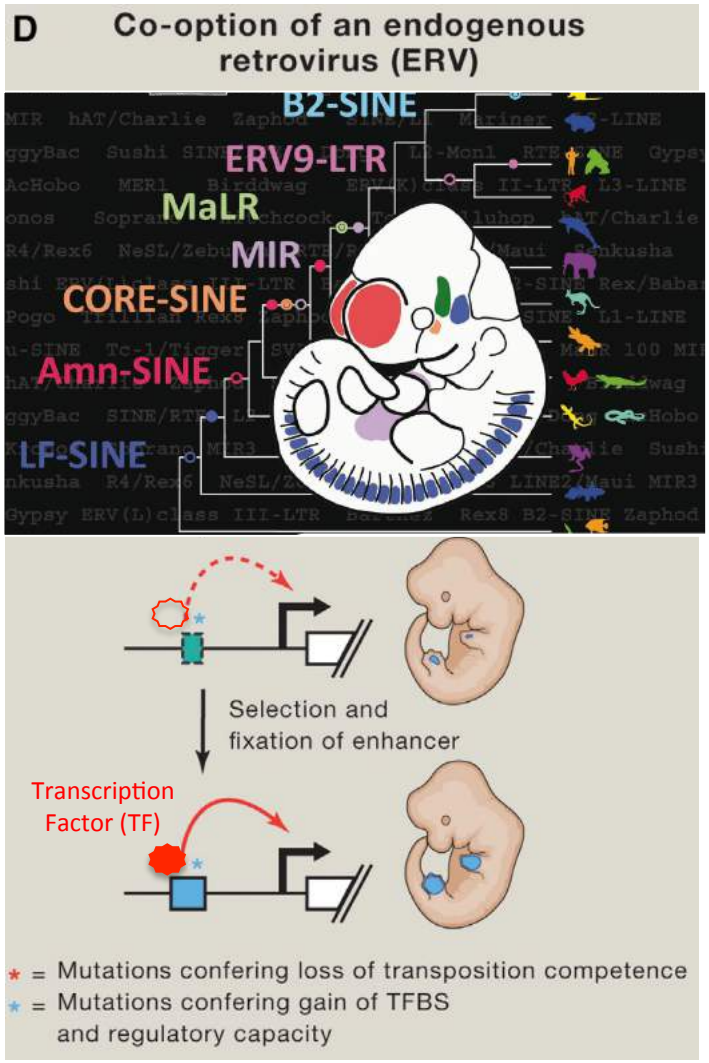
5. Some TEs (or their relics) have been coopted for gene regulation and orchestration of a number of processes during early embryonic development.

TE Relics co-opted as Modulators of Gene Expression

When transcriptionally active, TEs not only produce transcripts, some of which can have long-range regulatory functions, but can also stimulate the expression of nearby genes through promoter or enhancer effects.

TE-derived sequences and the evolution of Regulatory Networks

Next week:
COURS V



ERE-mediated, tissue-specific expression during early embryogenesis.

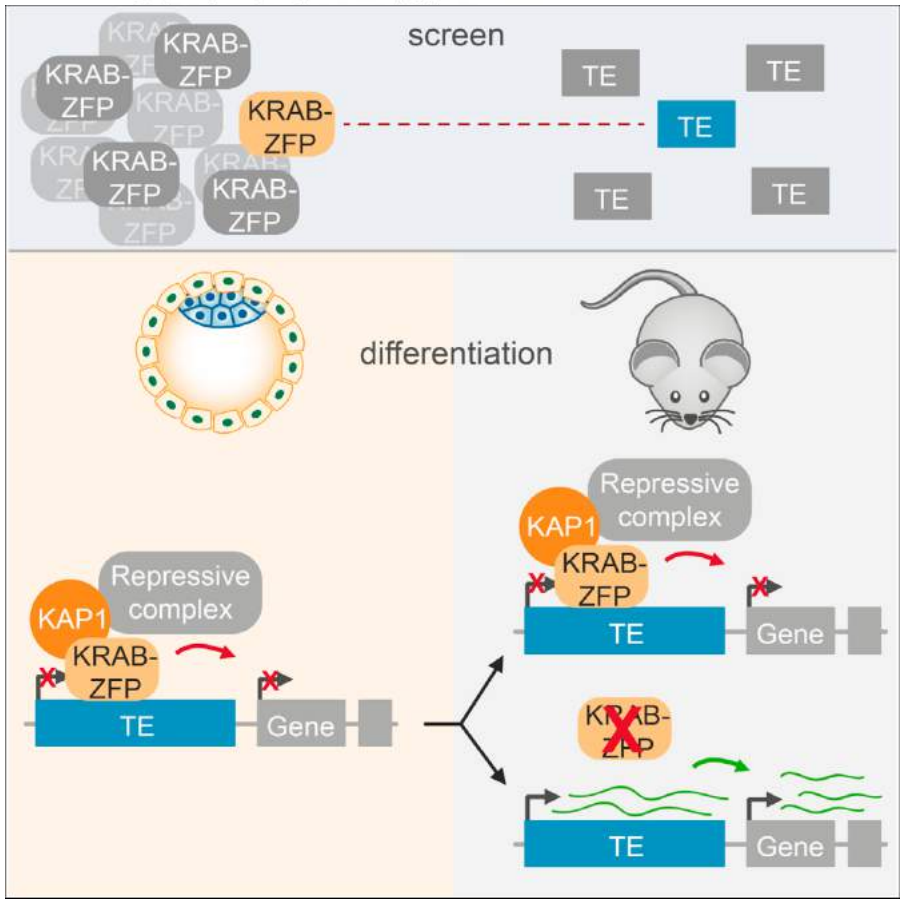
In human embryonic stem (ES) cells, 30% of transcripts are ERE-associated.
Fort et al. 2014, Lu et al. 2014, Santoni et al. 2012

Long et al, 2016

TEs and their KRAB-ZFPs Controllers Regulate Gene Expression in Adult Tissues

KRAB-ZFPs and KAP1 are embryonic controllers of transposable elements (TEs) thought to irreversibly silence TEs. These modulators continue to control TE expression in adult tissues, where they also act to control expression of neighboring cellular genes.

- KRAB-ZFPs control TEs (ERVs and LINEs) during development
- Specific ZFPs regulate specific ERV subsets
- Not just in embryos but also in somatic cells via histone modifications
- KRAB-ZFPs/KAP1 target TE relics and regulate secondarily expression of neighbouring genes in adult tissues
- Therefore TE relics are truly "controlling elements" as first proposed by McClintock
- KRAB-ZFP targeting in early development period results in **DNA methylation**, while in differentiated tissues triggers **histone-based modifications** : easier to remove?



Ecco et al, Dev. Cell, 2016

COLLÈGE DE FRANCE 1530

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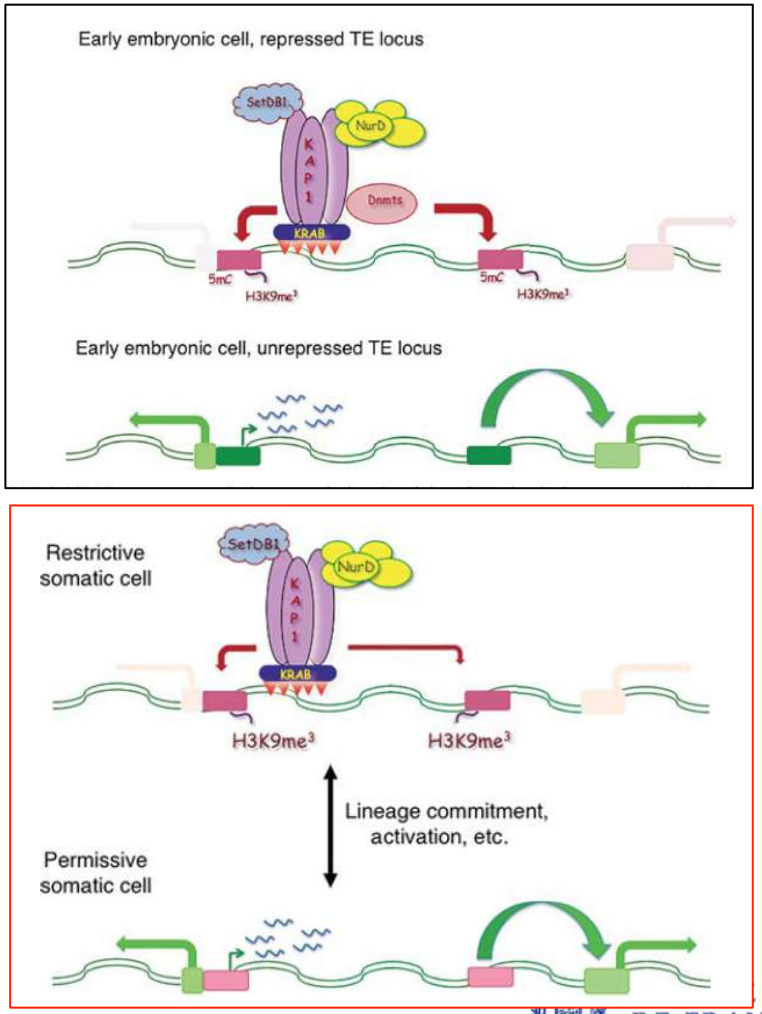

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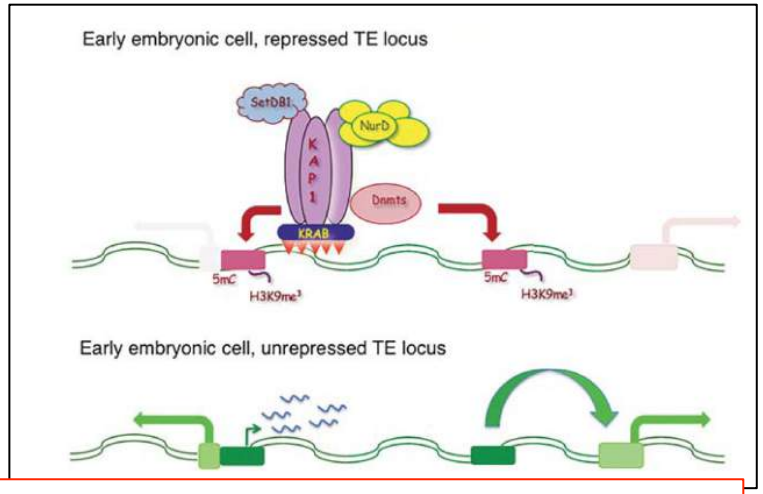


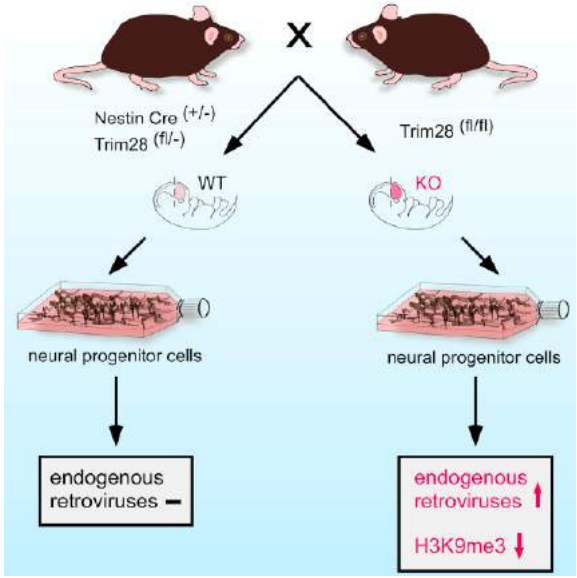
Deleting some KRAB-ZFPs leads to re-expression of certain TEs & nearby genes in ES cells and adult tissues
KAP1 binding is lost at the target sites
DNA Methylation is NOT affected in adult tissues
but H3K9 methylation is

=> Epigenetic plasticity at some TEs and nearby loci?

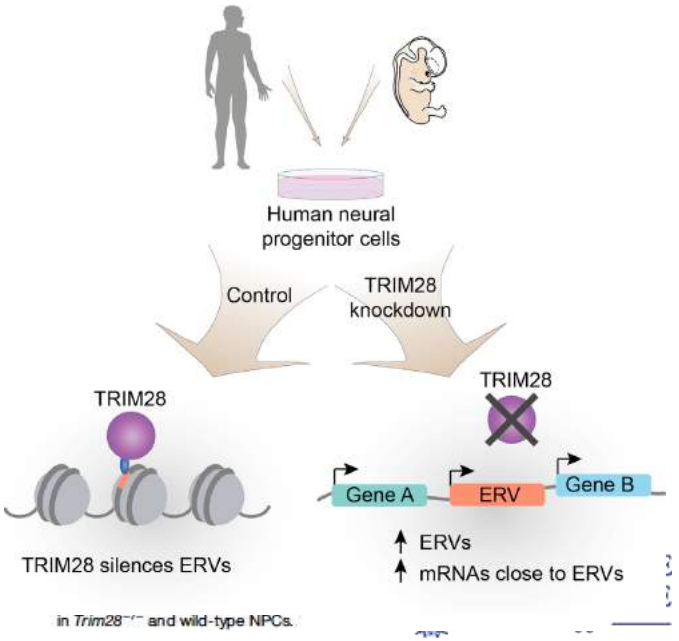
Ecco et al, Dev. Cell, 2016

TEs and their KRAB-ZFPs can Control Gene Expression via Histone Modifications in Adult Tissues

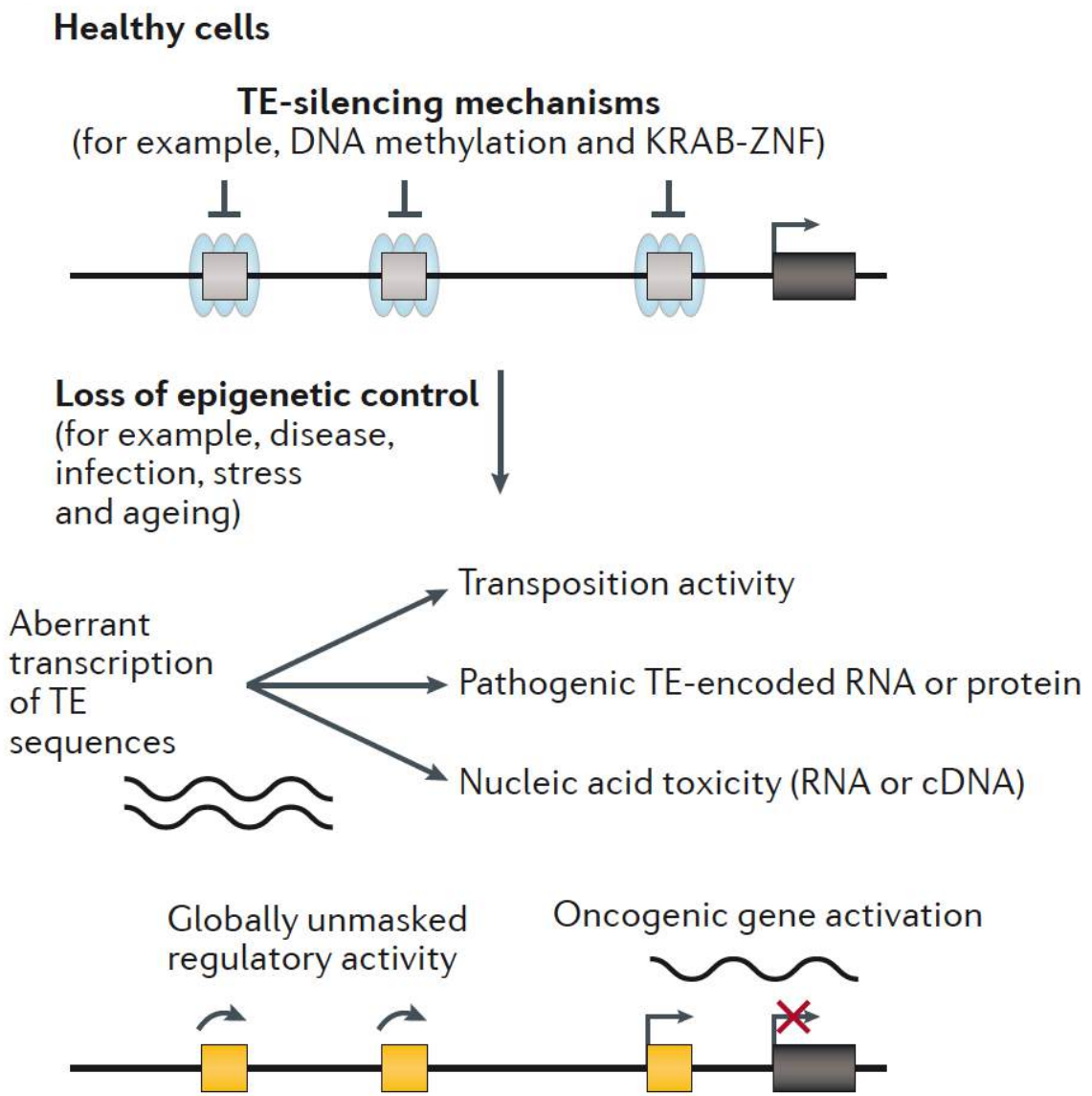
- Deletion of TRIM28 in NPCs results in transcriptional activation of ERVs (Fasching et al, 2015)
- ERVs are marked by H3K9me3 in NPCs, which is lost upon TRIM28 deletion
- Activation of ERVs in NPCs influences expression levels of nearby genes
- Activation of ERVs in NPCs results in the production of long noncoding RNAs
- **ERVs are controlled by TRIM28-mediated histone modifications in neural progenitor cells, suggesting a role for these elements in the control of transcriptional dynamics in the brain.**



- Stage- and region-specific expression of ERVs during human brain development (Brattas et al., 2017)
- TRIM28 binds to ERVs and induces hetereochromatin in human neural progenitor cells
- Knockdown of TRIM28 in hNPCs results in the upregulation of ERV expression
- Protein-coding genes located near upregulated ERVs are upregulated



TEs can be Aberrantly Reactivated to Promote Disease States



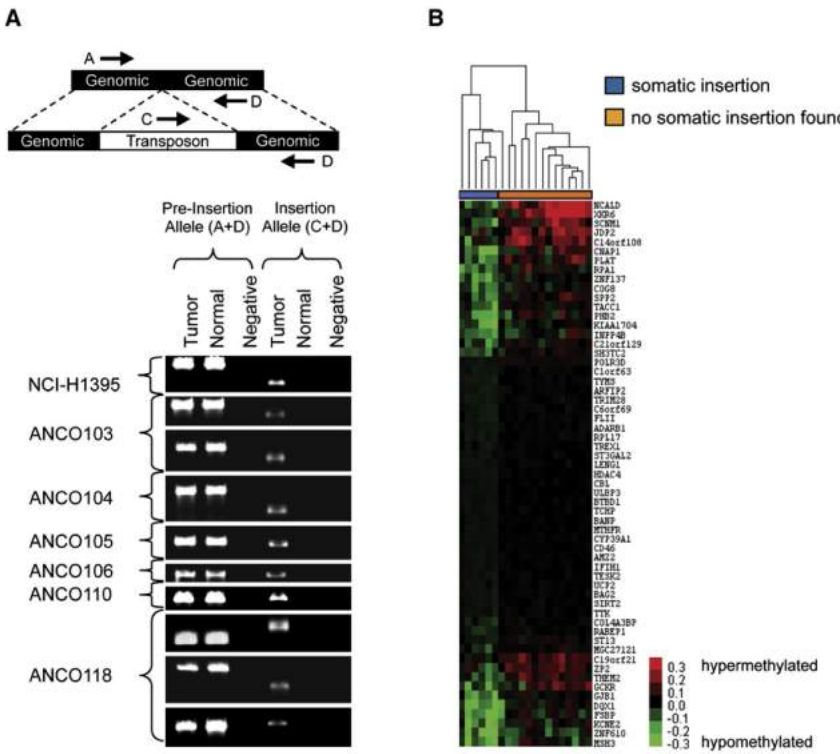
TEs and Disease:
Generators of potential mutations and epimutations

Natural Mutagenesis of Human Genomes by Endogenous Retrotransposons

Rebecca C. Iskow,[1,2,12] Michael T. McCabe,[3,6,13] Ryan E. Mills,[2,12] Spencer Torene,[2] W. Stephen Pittard,[7] Andrew F. Neuwald,[8,9] Erwin G. Van Meir,[4,5,6] Paula M. Vertino,[1,3,6] and Scott E. Devine[1,2,6,8,10,11,*]

- "Transposon-seq" methods were developed to find mobile element insertions in humans
- New germline retrotransposon insertions were identified in personal human genomes
- Tumor-specific somatic L1 insertions were uncovered in human lung cancer genomes
- Transposon mutagenesis is likely to have a major impact on human traits and diseases

R. C. Iskow et al., Natural mutagenesis of human genomes by endogenous retrotransposons. Cell 141, 1253–1261 (2010).



PCR Validation of Somatic Insertions and Identification of a Hypomethylation Signature in Tumors with New L1 Insertions

LINEs: new insertion rate estimated to be every 1 in 200 births

Alu repeats: 10^6 in human genome, 1 new Alu insertion for every 20 births

> 60 diseases so far due to Alu insertions in humans

TEs and Disease:
Generators of potential mutations and epimutations

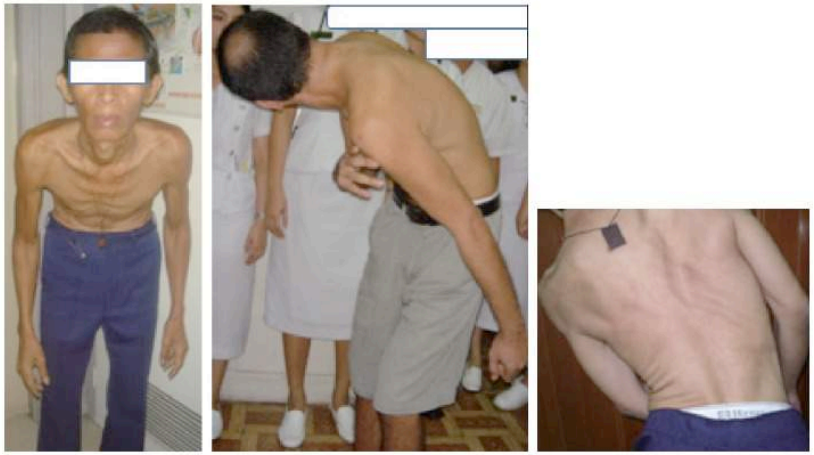
Table 1. Some human diseases linked to LINE and SINE insertions. The extensive role of LINEs and SINEs in the regulation of human gene expression suggests that they contribute to disease in as yet undiscovered ways.

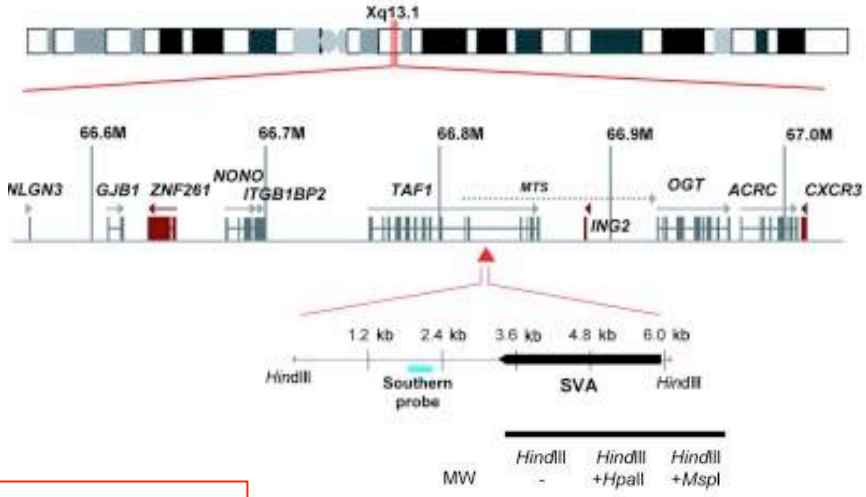
Effect of LINE or SINE insertion	Possible mechanism(s) of pathogenesis	Examples of associated diseases	Reference
Genomic deletions and rearrangements	LINE/SINE-mediated homologous recombination: DNA sequence loss; genomic instability	Prostate cancer, pyruvate dehydrogenase complex deficiency, leukemia, Alport syndrome, breast cancer	(*83*)
		Hereditary nonpolyposis colorectal cancer, Von Hippel–Lindau disease	(*86*)
Disruption of protein-coding sequences	Aberrant protein production; nonsense-mediated mRNA decay (NMD)	Hemophilia B, breast cancer, colon cancer, neurofibromatosis type 1	(*83*)
Altered DNA methylation	Increased expression of LINE and SINE RNA	Early event in many cancers	(*86*)
Altered pre-mRNA splicing	Aberrant protein production; NMD	Fukuyama-type congenital muscular dystrophy, neurofibromatosis type 1, hemophilia A	(*83*)
		Neurofibromatosis type 1, hemophilia A, breast cancer, Coffin-Lowry syndrome	(*84*)
Altered 3′-end formation	Premature transcription termination; altered protein production; NMD; altered mRNA stability, localization, or translatability	X-linked retinitis pigmentosa	(*83*)
Altered mRNA stability	Reduced protein production; altered temporal and/or spatial gene expression	X-linked dilated cardiomyopathy	(*83*)
		Hemophilia A, hereditary nonpolyposis colorectal cancer, hyper–immunoglobulin M syndrome	(*84*)
Sites of A-to-I editing	Loss of ADAR editing of target sites, possibly at *Alu* elements	Amyotrophic lateral sclerosis (ALS), astrocytoma, metastatic melanoma, Aicardi-Goutières syndrome, hepatocellular carcinoma	(*100*)

TEs and "Auto-Epigenetic" Disease

Human pathology: X-linked dystonia parkinsonism (Philippines)

Early onset Parkinson disease and distonia in male patients. Transposition of an SVA sequence in the *TAF1* gene: hypermethylation and downregulation in dystonic patient brains



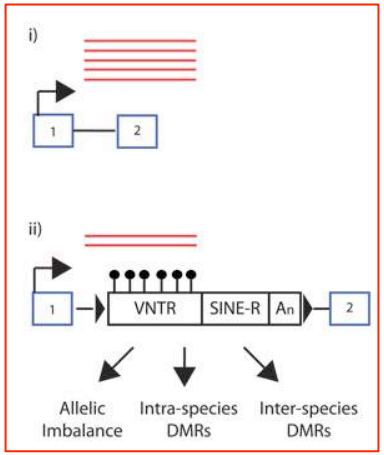


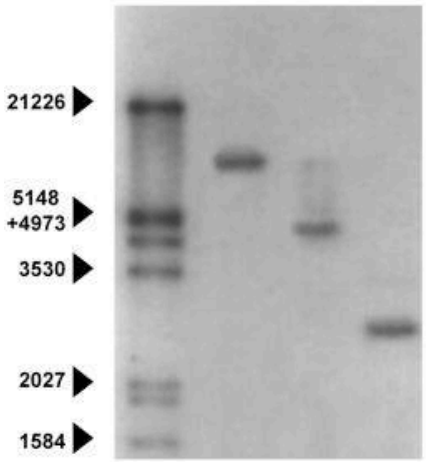
SINE-VNTR-Alus (SVA): composite, non-autonomous hominid specific retrotransposons, associated with disease in humans.

SVAs are evolutionarily young and presumably mobilized by LINE-1 reverse transcriptase in trans.

Reduced TAF1 mRNA expression in the caudate nucleus of XDP patients was associated with hyper-DNA methylation of the SVA as indicated by *HpaIII/ MspI* restriction analyses (Makino et al, 2007).

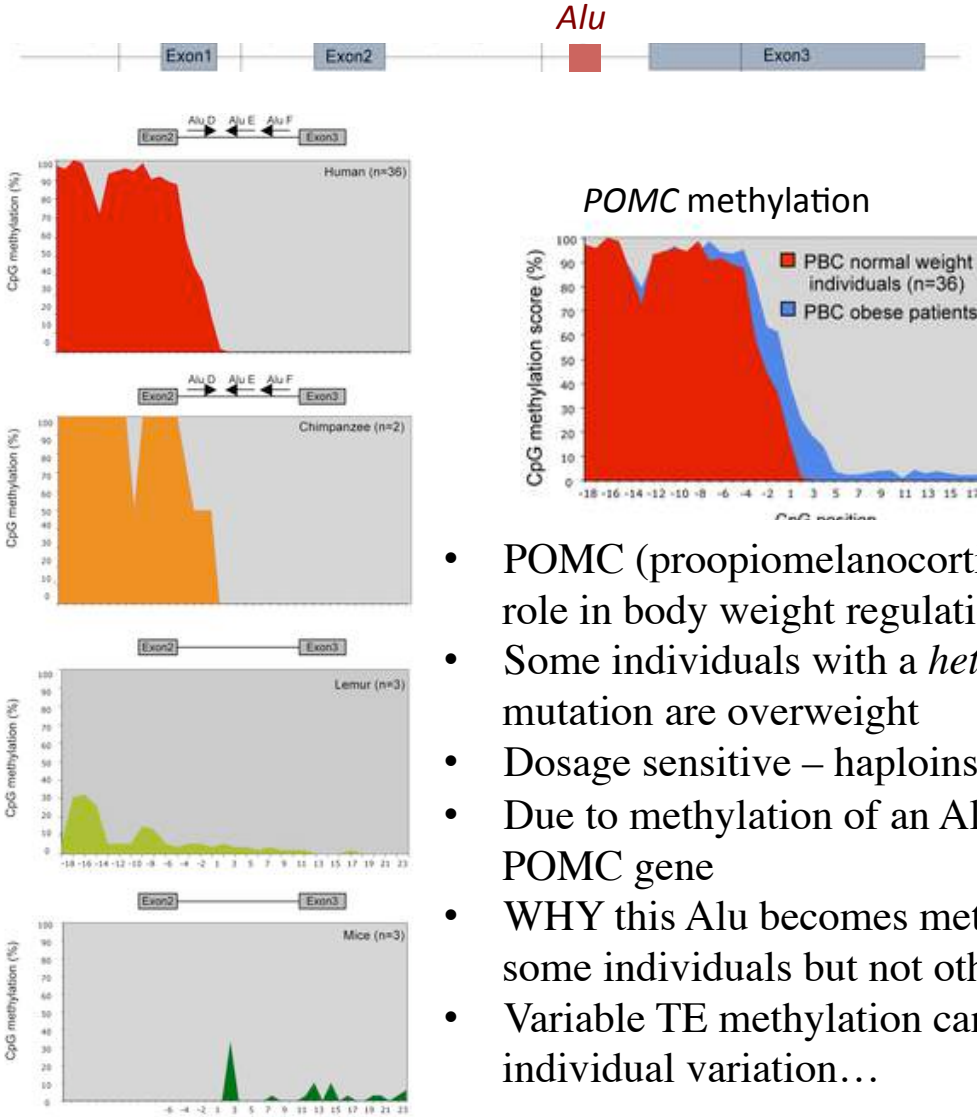
However degree and nature of disruption to TAF1 expression by the SVA not yet validated functionally (see Muller et al, 2007)





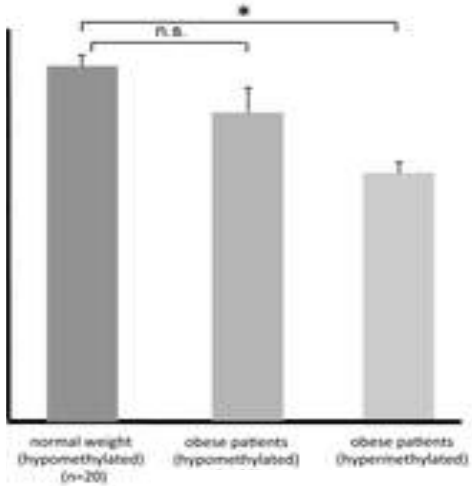
TEs and "Auto-Epigenetic" Disease

Childhood obesity: Presence of a primate-specific Alu sequence in *POMC* gene: hypermethylation & down-regulation in obese patients



POMC methylation

POMC expression



Normal weight

Obese Hypo methylation

Obese HYPER methylation

- POMC (proopiomelanocortin) plays key role in body weight regulation
- Some individuals with a *heterozygous* mutation are overweight
- Dosage sensitive – haploinsufficient?
- Due to methylation of an Alu within POMC gene
- WHY this Alu becomes methylated in some individuals but not others is unclear
- Variable TE methylation can lead to intra-individual variation…

Mobile Genetic Elements, 2:4, 197-201

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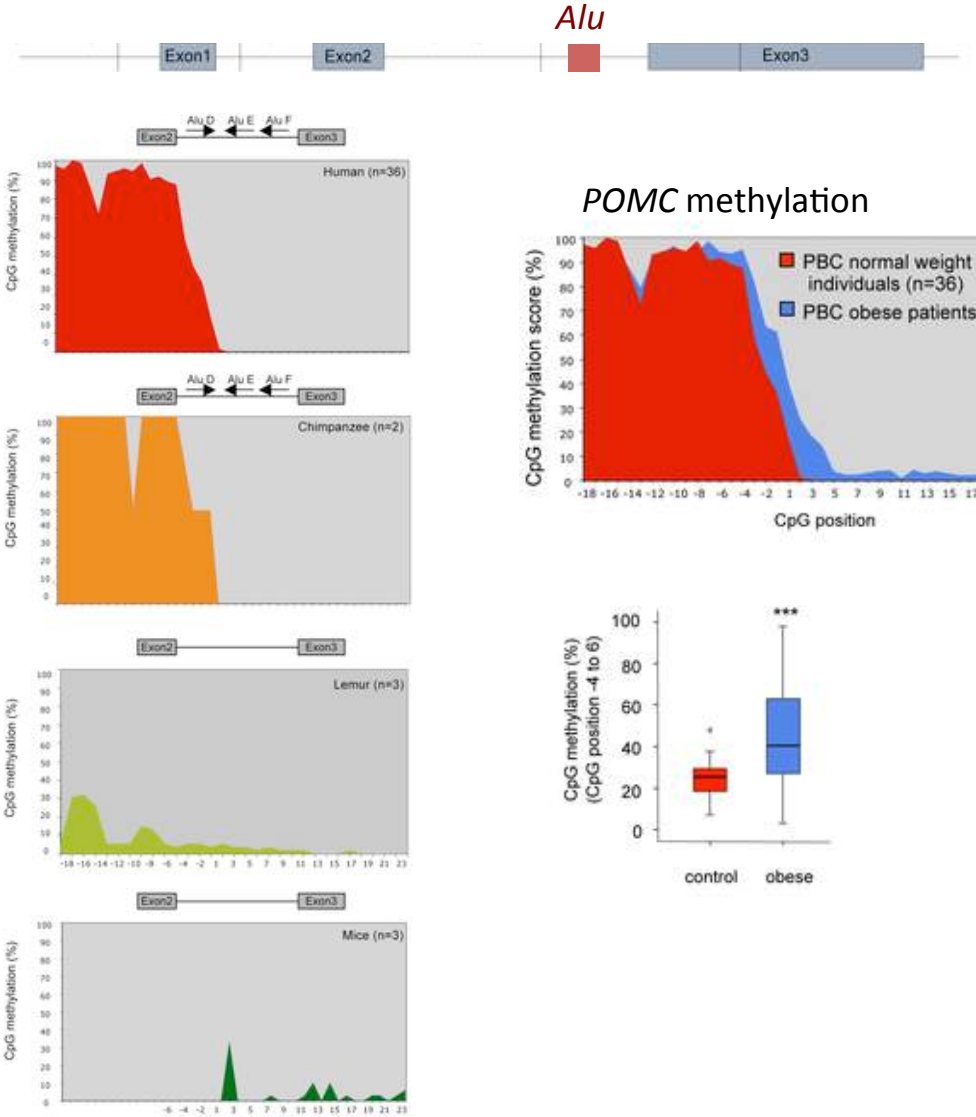

POMC methylation

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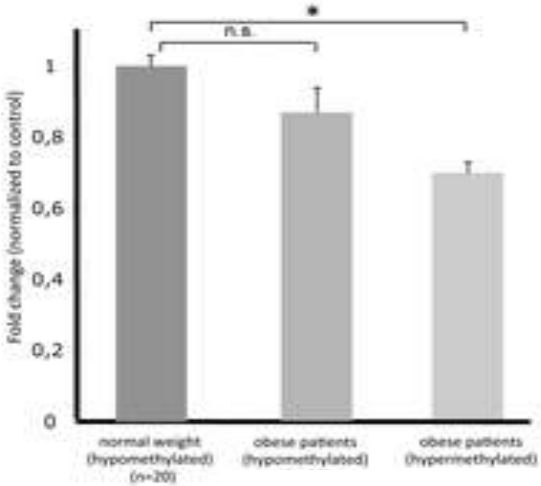

Normal weight

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Obese HYPER methylation

E. Heard, February 6th, 2017

Mobile Genetic Elements, 2:4, 197-201

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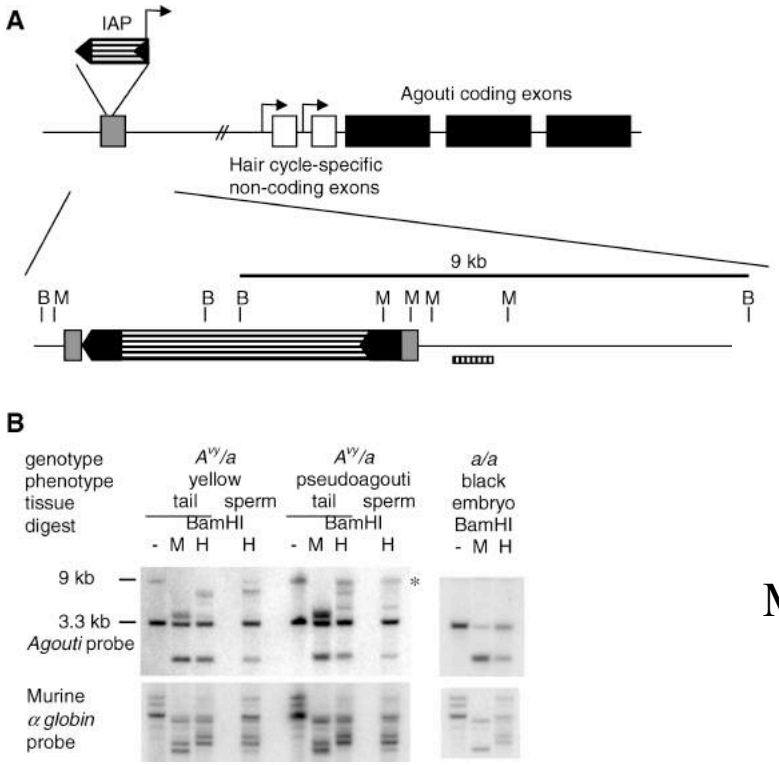
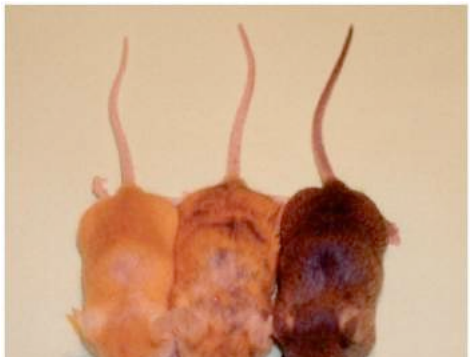
Transposable Elements (TEs) as Generators of Epigenetic Phenotypic Variation (*Epialleles*)

TEs attract epigenetic marking, providing phenotypic variation in absence of genotypic variation





And - these states can be influenced by maternal diet

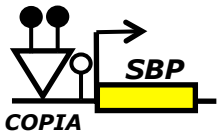
Cooney et al, *J. Nutr.* 2002
Maternal micronutrient supplementation can shift DNA methylation distribution and the corresponding fur phenotype at the population level in *Avy* mice

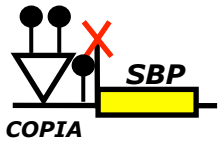
Waterland and Jirtle *Mol Cell Biol* 2003
Transposable elements: targets for early nutritional effects on epigenetic gene regulation.

Rakyan VK, Blewitt ME, Druker R, Preis JI, and E. Whitelaw. Metastable epialleles in mammals. Trends Genet 2002

Epigenetically Controlled TEs can also lead to Metastable states

cnr





(Manning et al, Nat Genet, 2006)

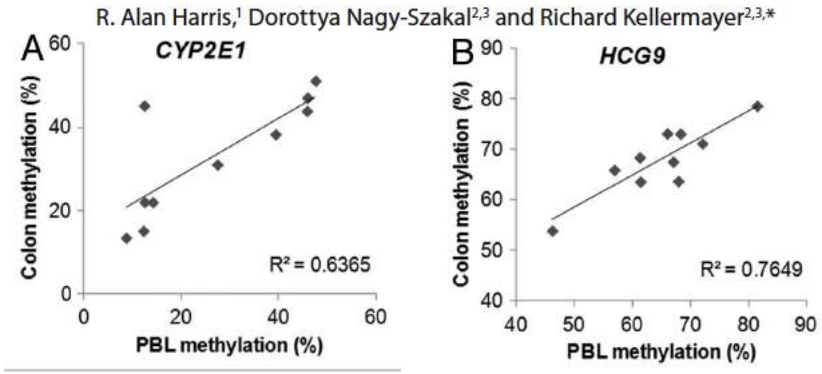
SBP encodes a transcription factor that allows ripening (red). In the *cnr* mutant a TE is integrated upstream of the promoter of *SBP*. The TE is constitutively methylated but its methylation can spread to the promoter of the gene and correlates with its silencing preventing ripening (yellow).

Silencing is **metastable** in somatic tissues – but fully stable through meiotic transmission.

Epigenetics 8:2, 157–163; February 2013; © 2013 Landes Bioscience

Human metastable epiallele candidates link to common disorders

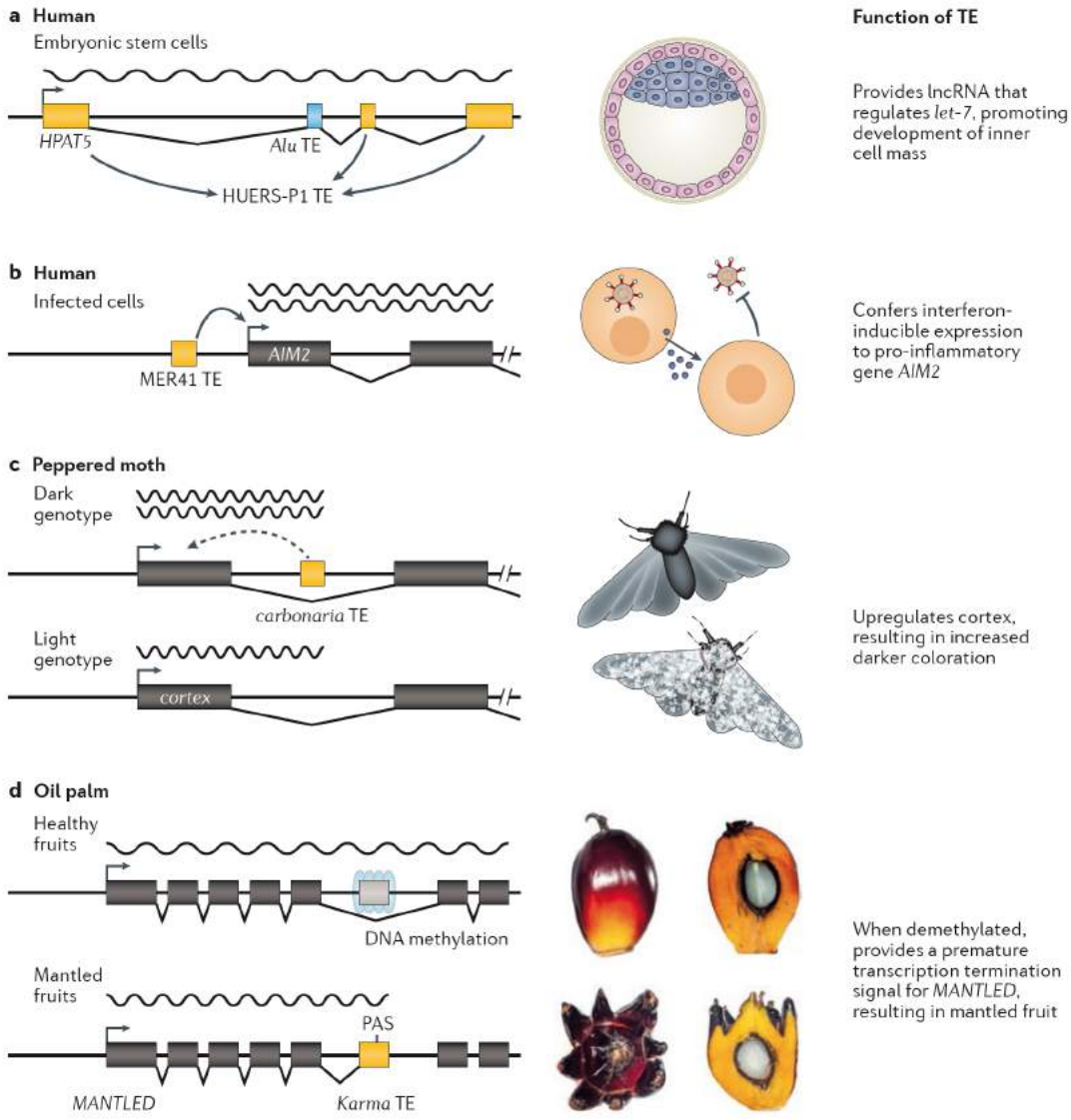
R. Alan Harris,[1] Dorottya Nagy-Szakal[2,3] and Richard Kellermayer[2,3,*]



Infinium HumanMethylation450 BeadChip kits in a 2-tissue parallel screen to examine DNA methylation at

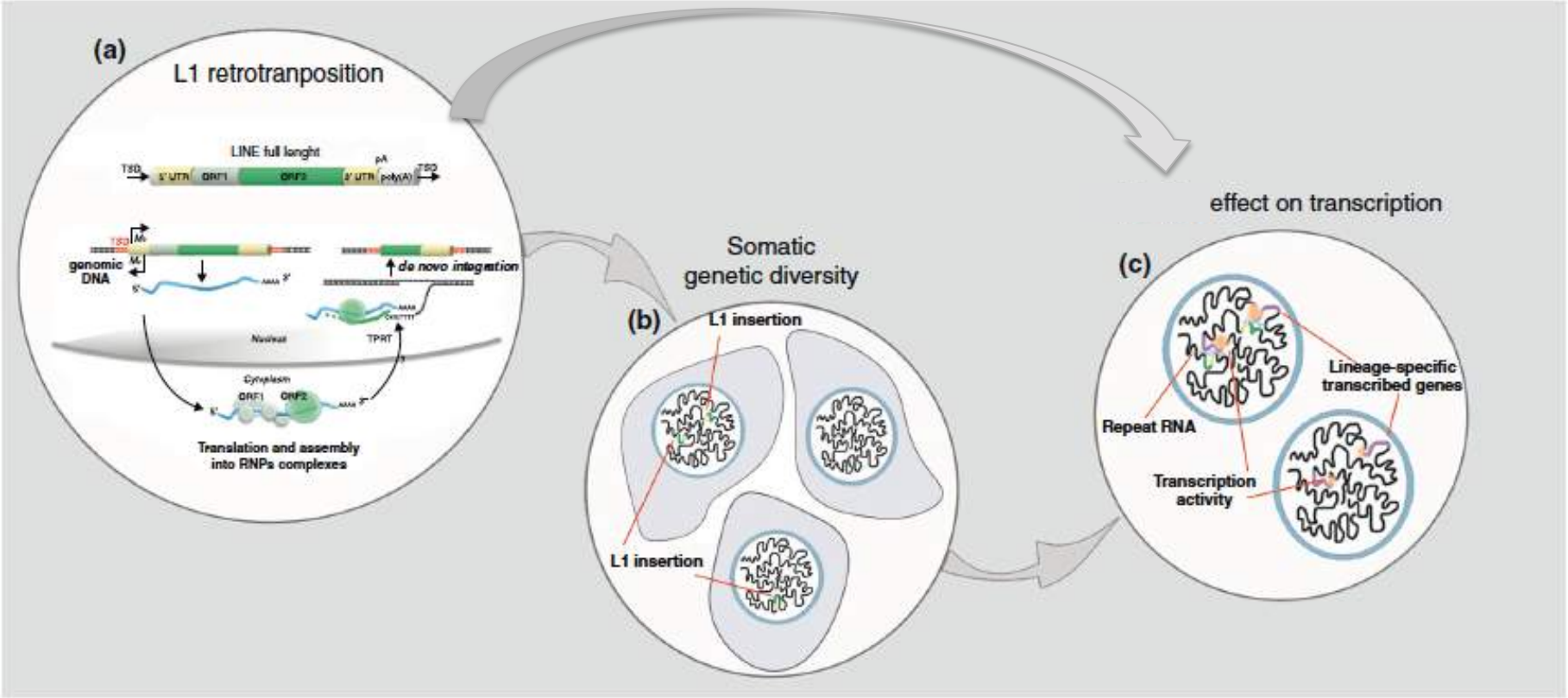
- "Metastable" CpG methylated site variation in disease-associated genes (Parkinson, Bipolar disorder)
- No clue as to *cause* of variability
- No link to TEs (but did not look)
- Big challenge: to know the actual contribution epigenetics makes to phenotypic states in humans due to extreme genetic heterogeneity. Studies in monozygotic twins required…

Phenotypes driven by TE regulatory activity via both genetic and epigenetic control



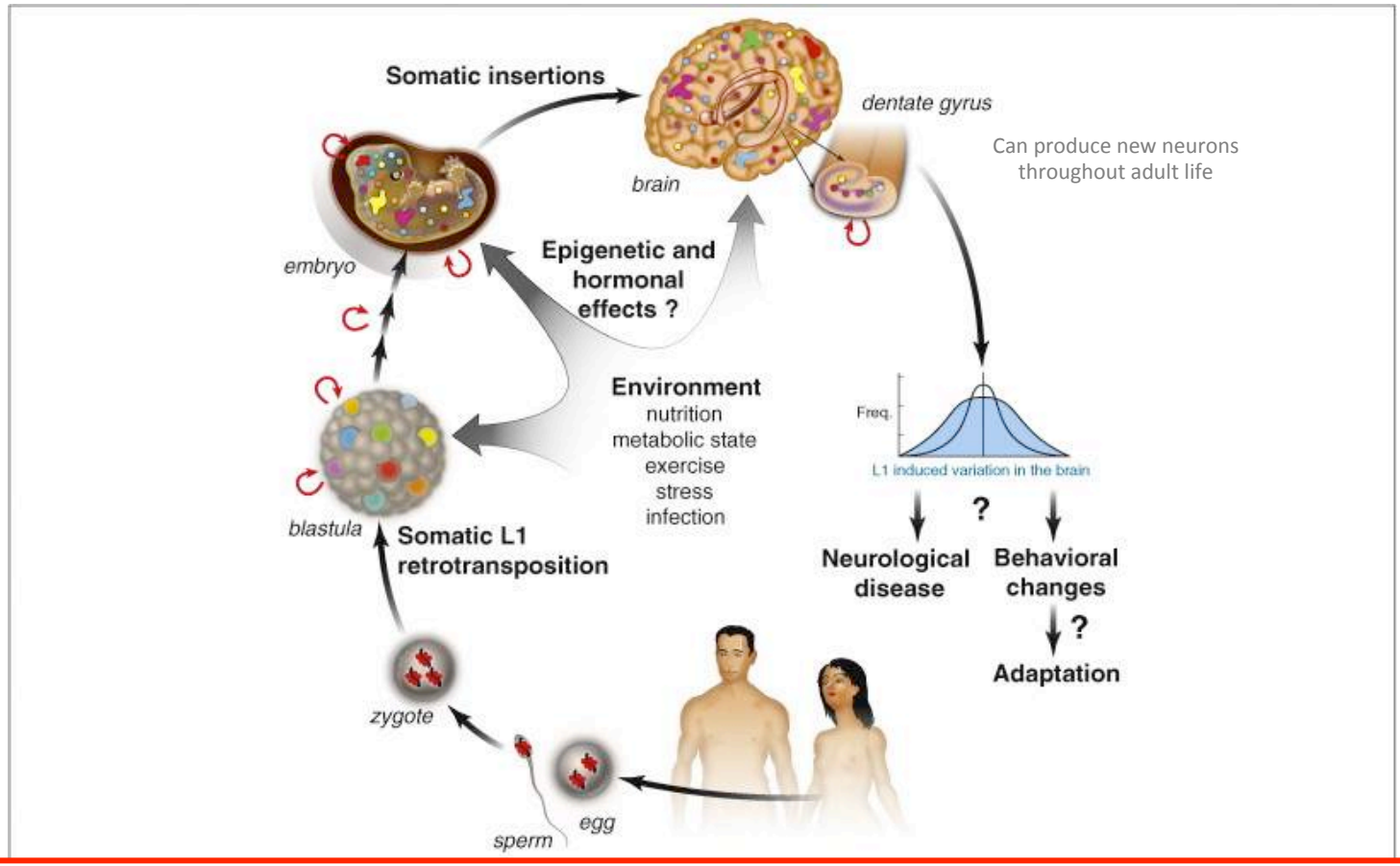
Chuong et al, 2016

Transposable Elements (TEs) as Generators of Genetic and/or Epigenetic Variation in the **Soma**



Bodega and Orlando, Current Opinion in Cell Biology 2014, 31:67–73

Transposable Elements (TEs) as Generators of Genetic and/or Epigenetic Variation in the **Brain**



- TEs actively retrotranspose during neurogenesis: genomic diversity between neurons.
- TE expression and retrotransposition can be affected by stress: TEs can lead to changes in cellular phenotype => active transposition may be advantageous in coping with stress?
- TE-driven expression/mobility may be *mis-regulated* in certain neurological disorders, eg Rett syndrome and schizophrenia.

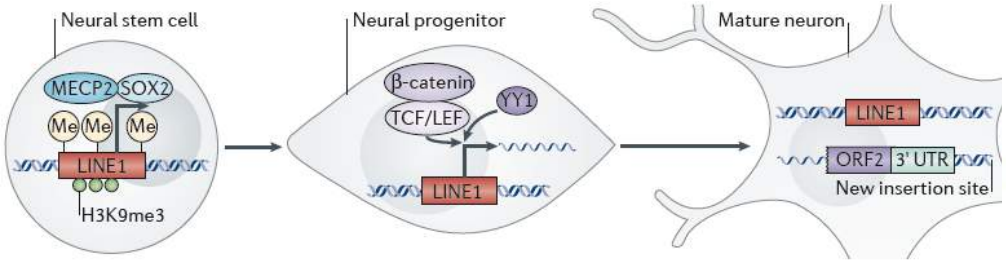
Transposable Elements (TEs) as Generators of Neuronal Mosaicism in Mice and Humans

Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition

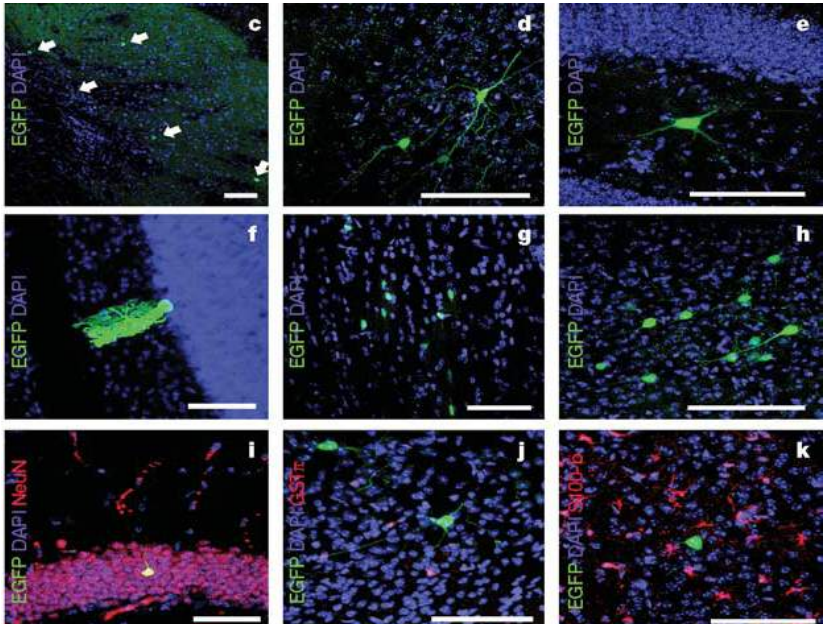
Alysson R. Muotri[1]*, Vi T. Chu[1]*†, Maria C. N. Marchetto[1], Wei Deng[1], John V. Moran[2] & Fred H. Gage[1]

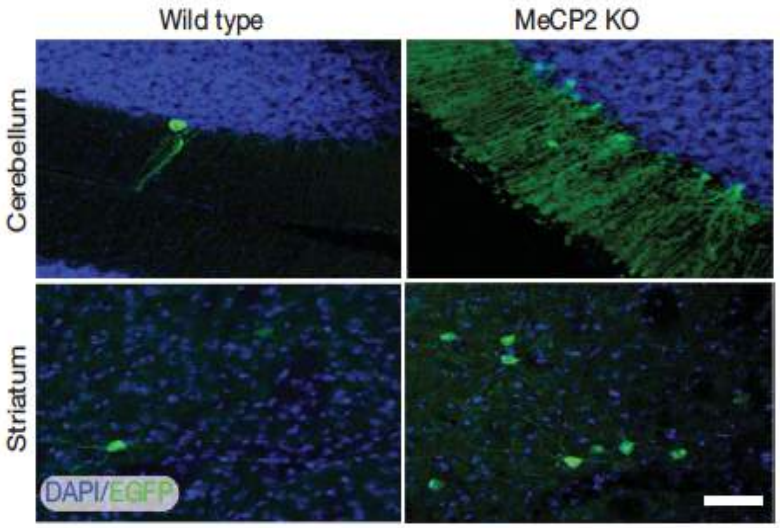
- An engineered human LINE-1 can retrotranspose in neuronal precursors derived from rat hippocampus neural stem cells.
- Resulting retrotransposition events can **alter the expression of neuronal genes** and **influence neuronal cell fate *in vitro*** => Role? Or accidents?
- Retrotransposition of a human L1 in transgenic mice results in neuronal somatic mosaicism.
- Neuronal genomes are highly DYNAMIC

Mechanism? DNA methylation and H3K9me3 repression
Sox2/HDAC1 repressor complex shifts to Wnt-mediated (TCF/LEF) transcriptional activator of LINEs
Mecp2 loss (Rett's syndrom) increases L1 reactivation (Muotri et al, Nature 2010)



L1 retrotransposition detection in the brains of transgenic mice





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Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition

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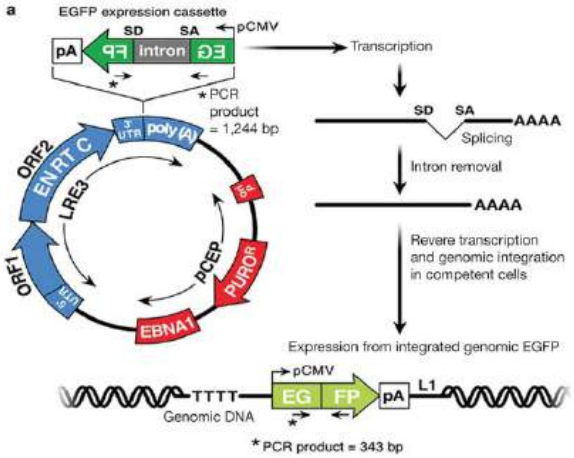
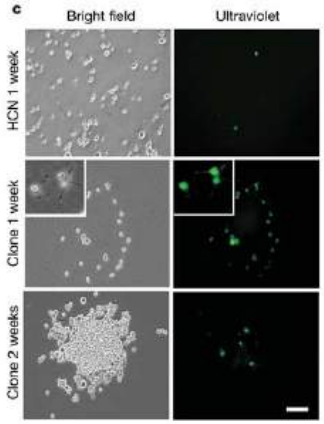
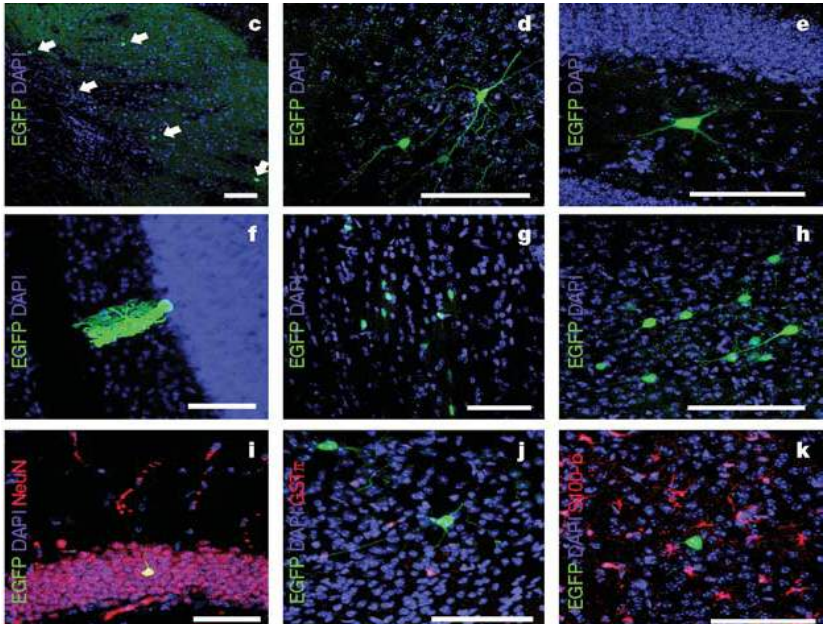
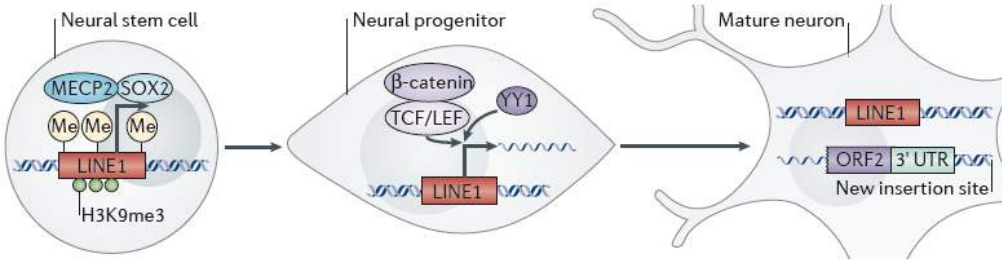
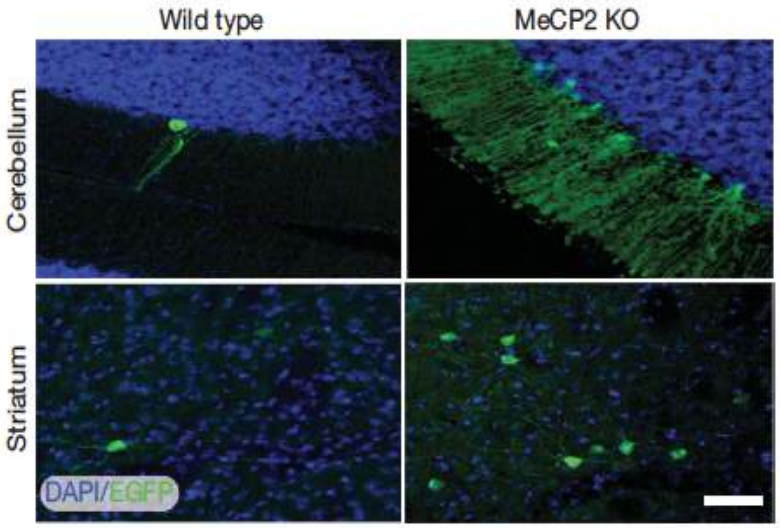




L1 retrotransposition detection in the brains of transgenic mice



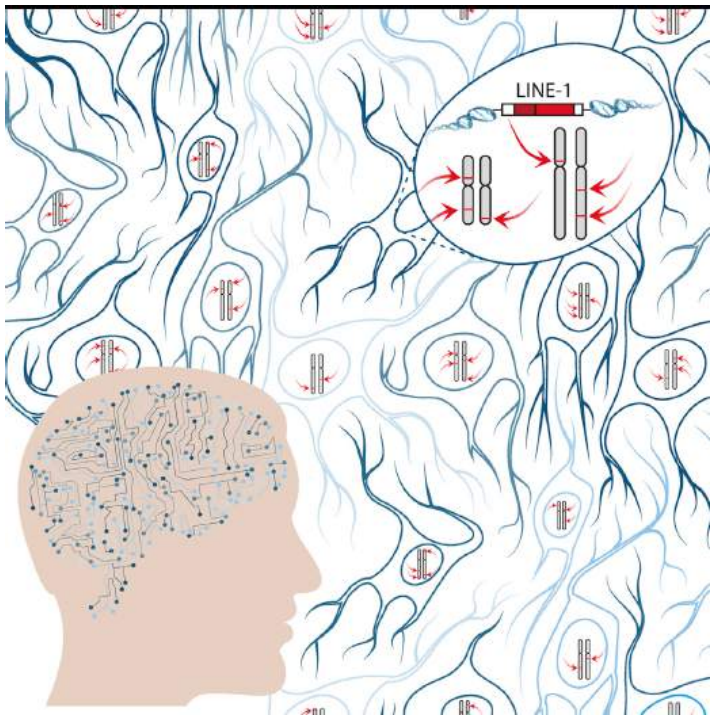




High-throughput sequencing to identify endogenous brain-specific LINE1 insertions in post-mortem human tissue

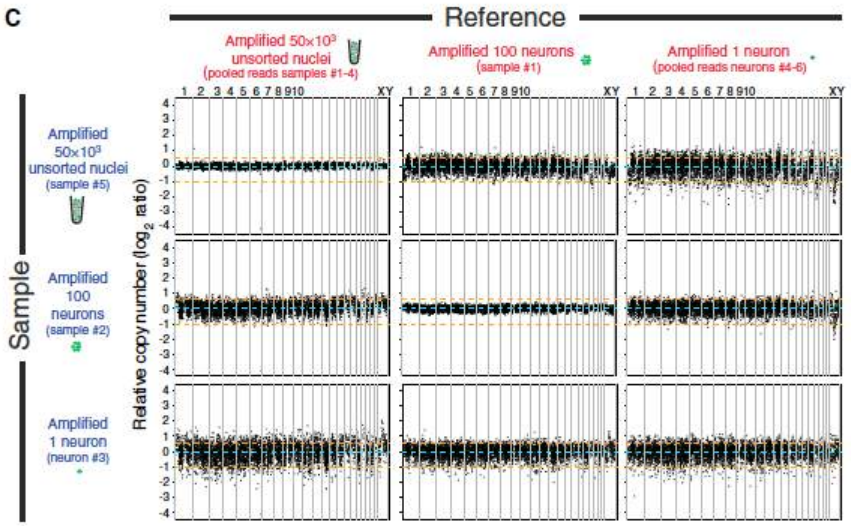
Ubiquitous L1 Mosaicism in Hippocampal Neurons

Kyle R. Upton,[1,6] Daniel J. Gerhardt,[1,6] J. Samuel Jesuadian,[1,6] Sandra R. Richardson,[1] Francisco J. Sánchez-Luque,[1] Gabriela O. Bodea,[1] Adam D. Ewing,[1] Carmen Salvador-Palomeque,[1] Marjo S. van der Knaap,[2] Paul M. Brennan,[3] Adeline Vanderver,[4] and Geoffrey J. Faulkner[1,5,*]



Single-Neuron Sequencing Analysis of L1 Retrotransposition and Somatic Mutation in the Human Brain

Gilad D. Evrony,[1,5,6,11] Xuyu Cai,[1,5,6,11] Eunjung Lee,[2,9] L. Benjamin Hills,[5,6] Princess C. Elhosary,[7] Hillel S. Lehmann,[5,6] J.J. Parker,[5,6] Kutay D. Atabay,[5,6] Edward C. Gilmore,[10] Annapurna Poduri,[3,7] Peter J. Park,[2,8,9] and Christopher A. Walsh[1,3,4,5,6,*]



Evrony, G. D. *et al*. *Cell* **151**, 483–496 (2012).
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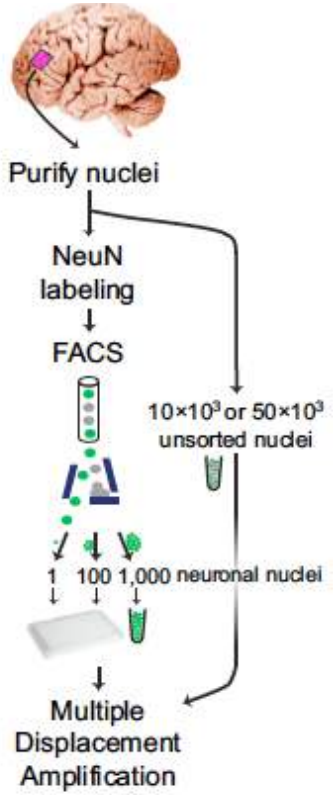

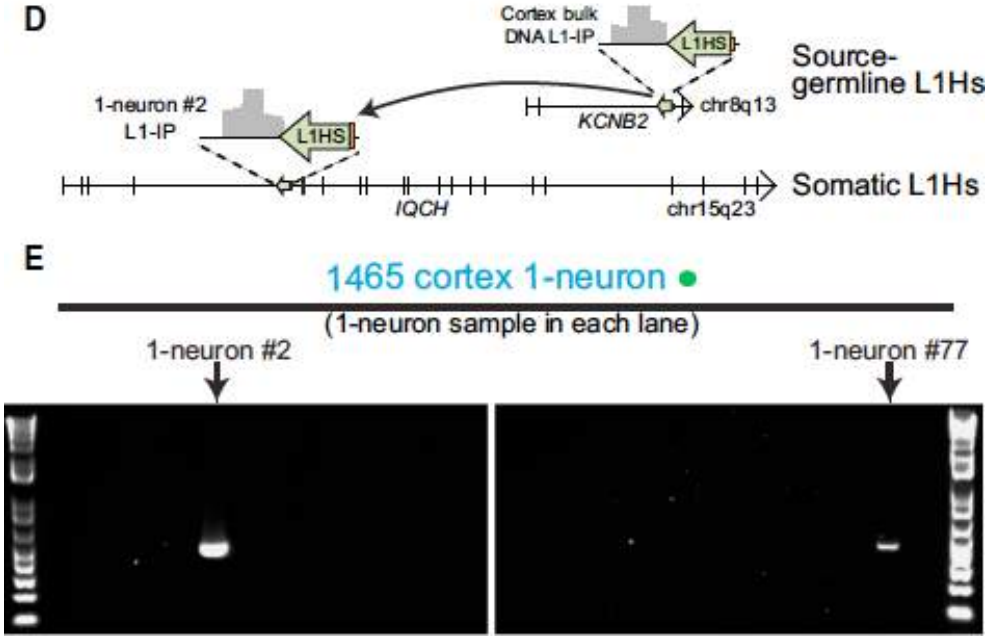

- Detect somatic L1Hs insertions in normal human brain.
- Very low-level mosaicism of this insertion and its detection only in cortical neurons suggest that it occurred during cortical development.
- The source full-length L1Hs on chromosome 8 from which the somatic insertion originated lies in intron of *KCNB2* gene – (not present in all individuals)

Evrony, G. D. *et al*. Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. *Cell* **151**, 483–496 (2012).

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Resolving rates of mutation in the brain using single-neuron genomics

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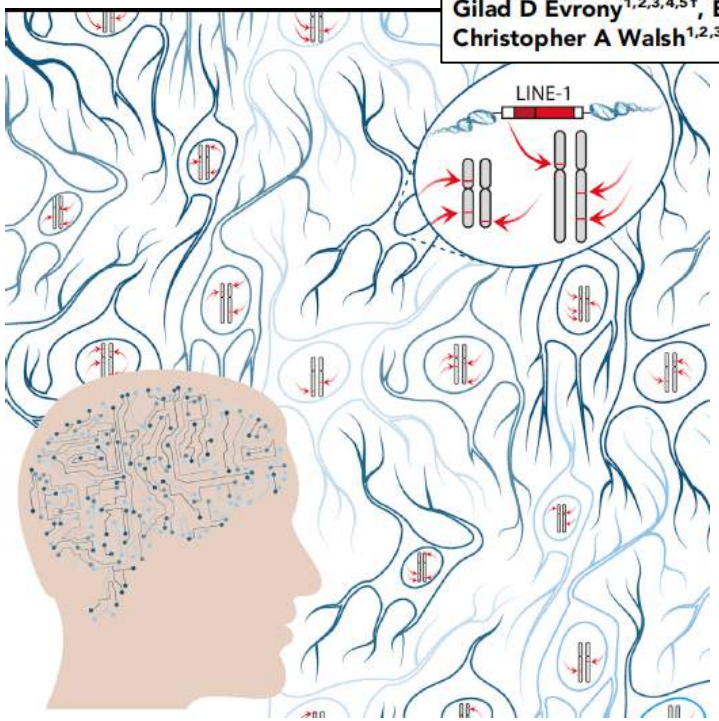

L1 insertion rates?

- Upton et al (2015) suggested that in the Hippocampus (neurogenic region) have up to 13.7 new L1 insertions/cell!
- Preferentially in sense orientation in expressed genes
- => L1 TEs could play a **role** in the healthy brain?
- However, other studies (Evrony et al, 2012; Evrony et al 2016, show fewer mutations, about 0.2 events/cells
- => **50 times less**

⇒ L1 mobility is more likely to be an occasional source of rare variation or disease, NOT an essential contributor to normal brain activity in humans.

⇒ And, L1 *expression* may be ubiquitous and have a big impact on gene activity and cell functions in the brain…

Evrony, G. D. *et al*. *Cell* **151**, 483–496 (2012).
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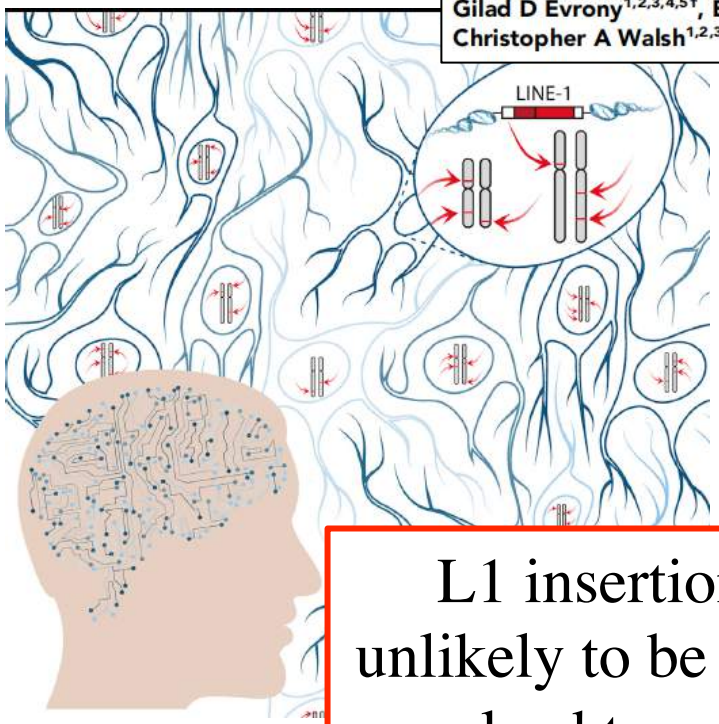

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L1 insertions are not "ubiquitous" and are unlikely to be regulatory - but they can certainly lead to variations between individuals.

Even with a conservative estimate of 1 insertion per 300 cells, the human brain would contain >100 million unique somatic insertions

source of
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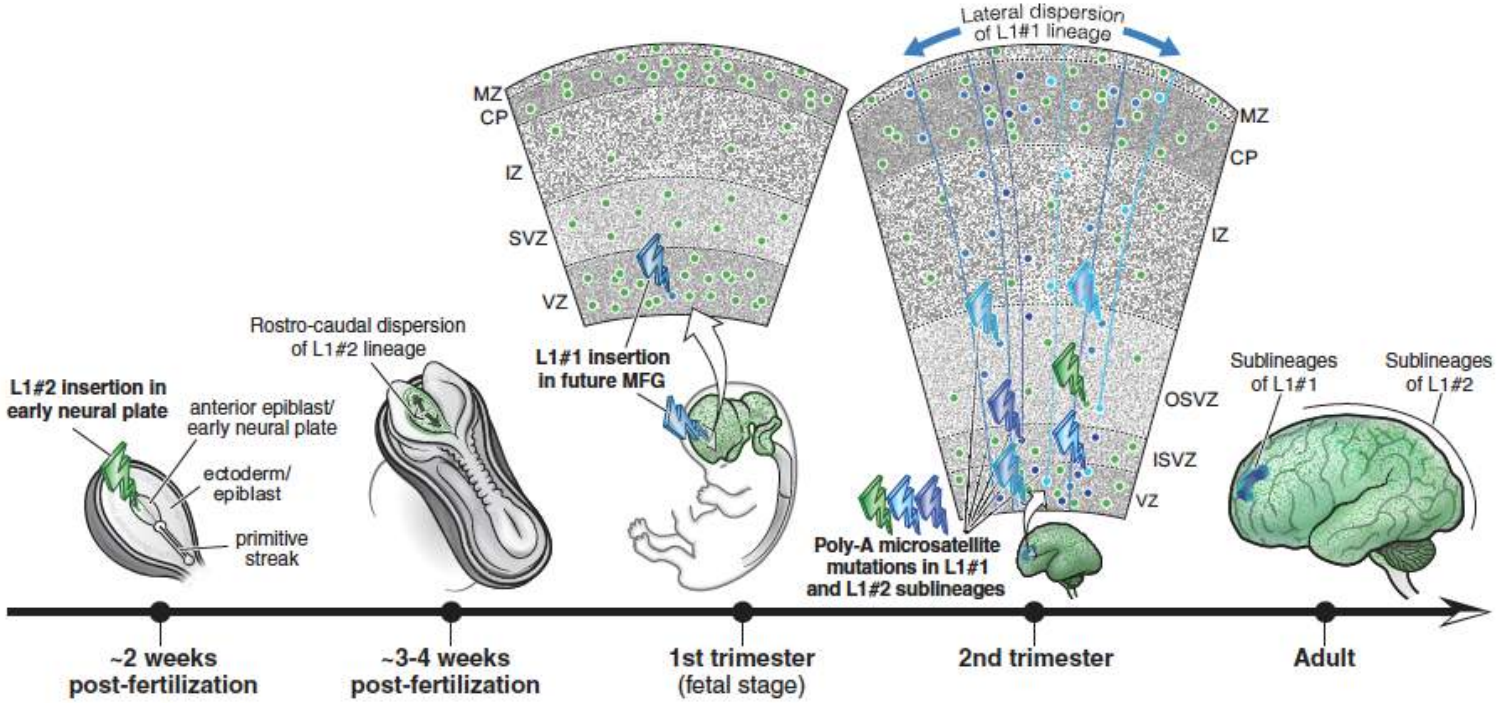
Evrony, G. D. *et al*. C
Baillie, J. K. et al. Nat
Upton K. et al. Cell 16

Lineage Tracing Mutation events in the Human Brain using LINE-1 insertions and internal mutations

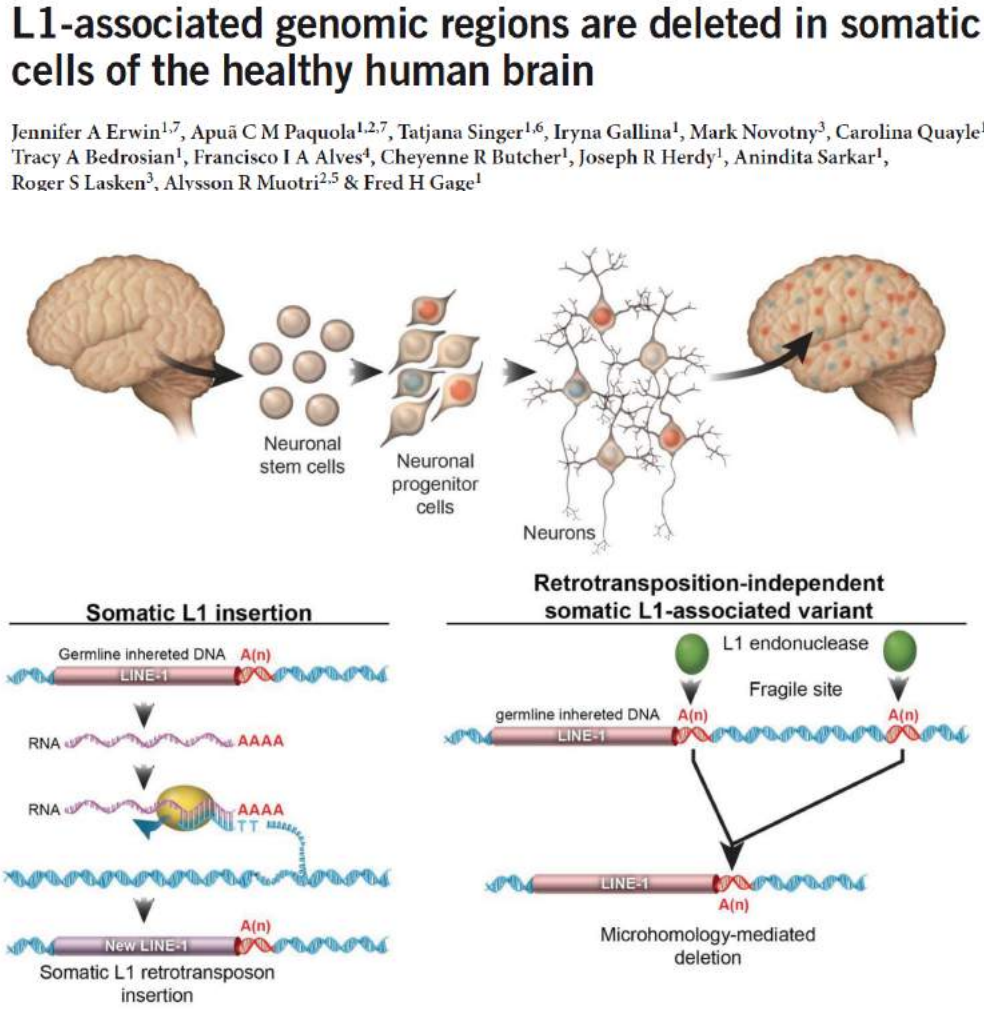
Cell Lineage Analysis in Human Brain Using Endogenous Retroelements

Gilad D. Evrony,[1,2,3,9] Eunjung Lee,[4,5,9] Bhaven K. Mehta,[1,2,3] Yuval Benjamini,[6] Robert M. Johnson,[7] Xuyu Cai,[1,2,3,8] Lixing Yang,[4,5] Psalm Haseley,[4,5] Hillel S. Lehmann,[1,2,3] Peter J. Park,[4,5,10,*] and Christopher A. Walsh[1,2,3,10,*]

- High-coverage whole-genome sequencing of **single neurons** from normal human brain
- Spatial tracing of cell lineages in human brain using somatic retrotransposon insertions
- Highly dynamic mutation of microsatellite repeats within insertions marks sublineages
- Somatic mutations reveal patterns of clonal dispersion and focal mutation in normal brain



L1-associated genomic regions are deleted in somatic cells of the healthy human brain



- Human brain is a **mosaic** of varied genomes.
- Using machine learning-based, single-cell sequencing, somatic L1-associated variants (SLAVs) identified – of two classes:
- L1 retrotransposition insertions
- Retrotransposition-independent L1-associated variants.
- Some SLAVs comprise somatic deletions generated by L1 endonuclease cutting activity.
- Retrotransposition-independent rearrangements in from inherited L1s show deletion of proximal genomic regions, resolved by microhomology-mediated repair
- => L1-associated genomic regions are hotspots for somatic copy number variants in the brain and contribute to somatic mosaicism.
- SLAVs are present in crucial neural genes, such as *DLG2* (also called *PSD93*), and **affect 44–63% of cells of the cells in the healthy brain**

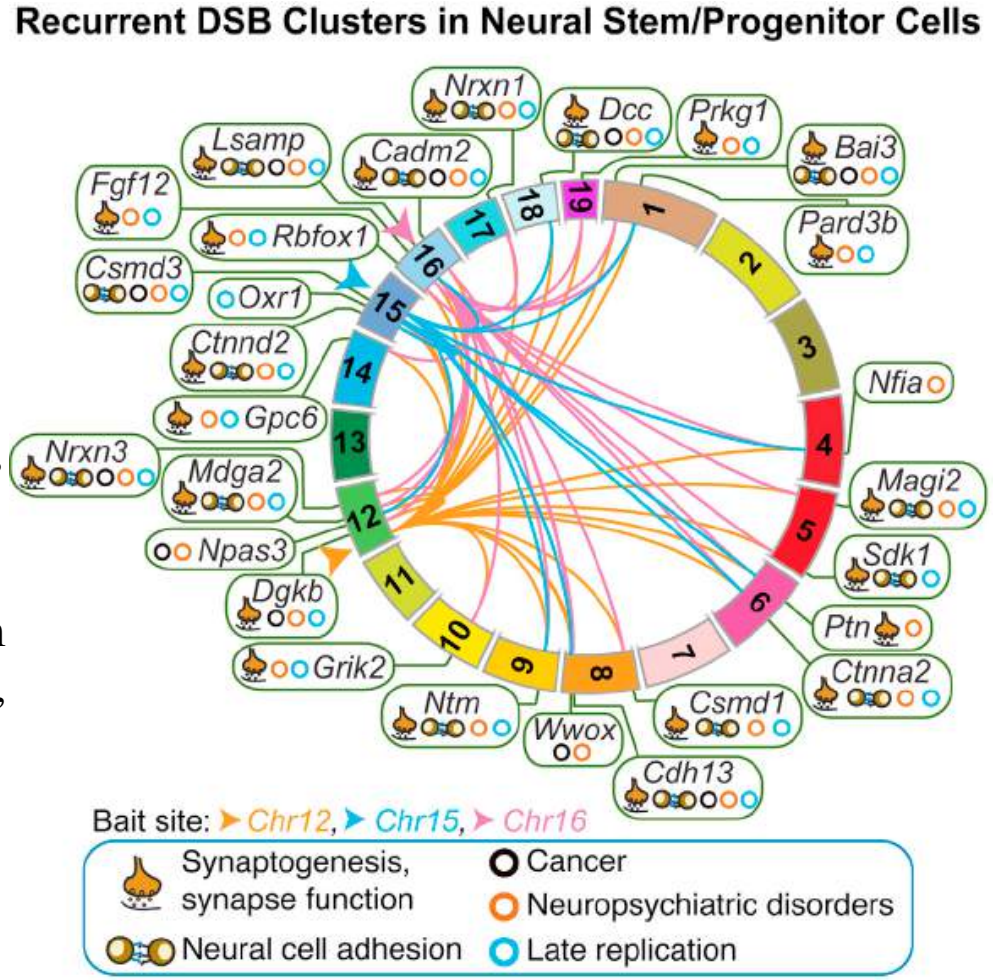
Nature Neuroscience 19, 1583–1591 (2016)

Many sources of somatic mosaicism in neurons!

Neural stem and progenitor cells undergo very frequent DNA breaks in a very restricted set of genes involved in neural cell adhesion and synapse function.

Long Neural Genes Harbor Recurrent DNA Break Clusters in Neural Stem/Progenitor Cells

Pei-Chi Wei,[1,2,3,4] Amelia N. Chang,[1,2,3,4] Jennifer Kao,[1,2,3] Zhou Du,[1,2,3] Robin M. Meyers,[1,2,3] Frederick W. Alt,[1,2,3,*] and Bjoern Schwer[1,2,3,*]



The source of widespread low-level DSBs in NSPCs is not yet known.

Such DSBs might arise from various endogenous sources, including replicative, transcriptional, or oxidative stress

E. Heard, 2017

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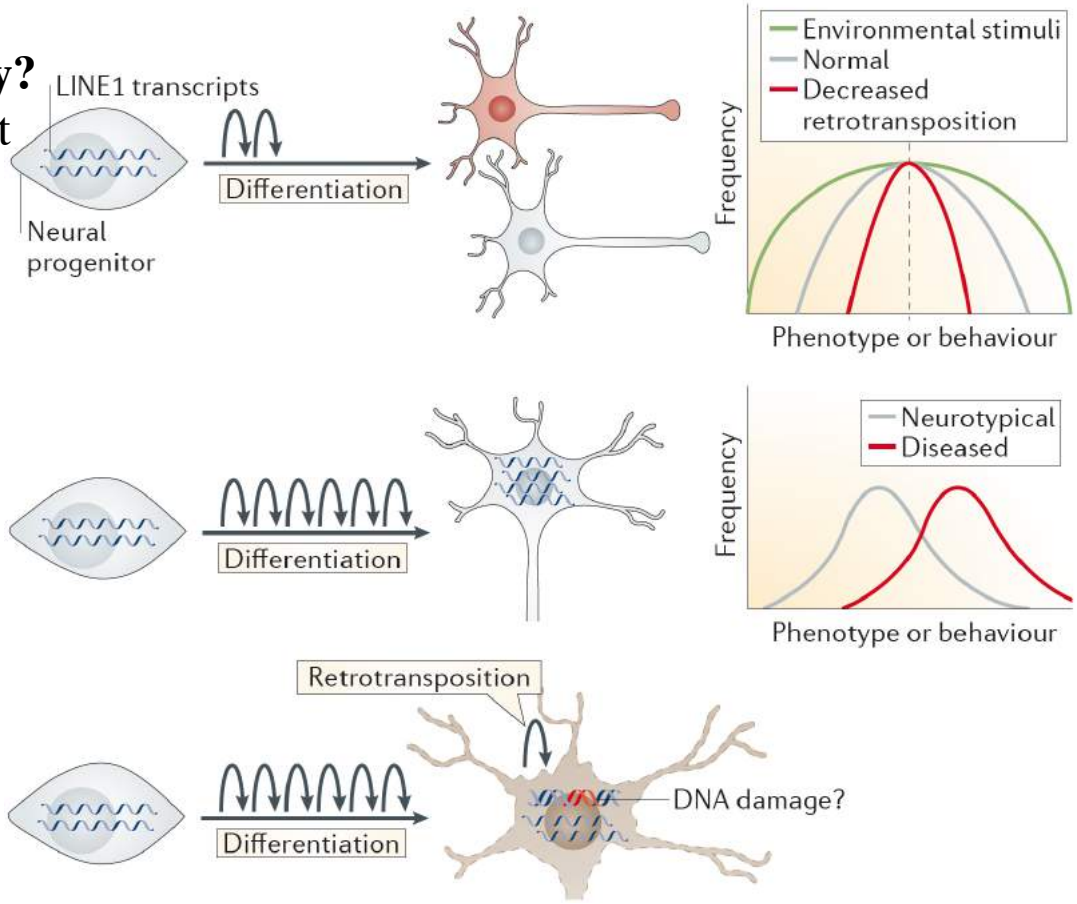
Effects of somatic mosaicism in neurons

- LINE1 elements are not only expressed in the mouse, human and *Drosophila melanogaster* brain but are also actively retrotransposed in these species
- Mobilization of LINE1 retrotransposons generates neuronal somatic mosaicism

What is its role – if any?
Hippocampal-dependent learning and memory?
Stress Responses?
Diseases?



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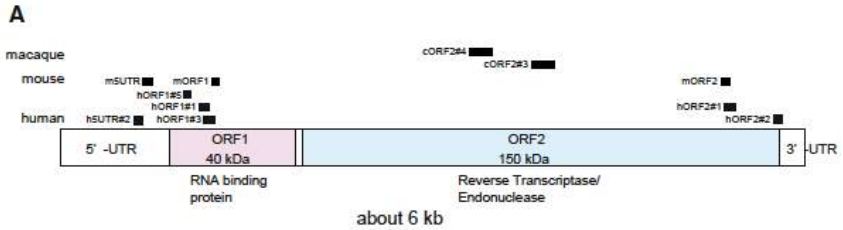
Evrony et al. Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. *Cell* **151**, 483–496 (2012)
Perrat, P. N. et al. Transposition-driven genomic heterogeneity in the Drosophila brain. Science 340, 91–95 (2013)

Increased L1 retrotransposition in the neuronal genome in schizophrenia

Increased L1 Retrotransposition in the Neuronal Genome in Schizophrenia

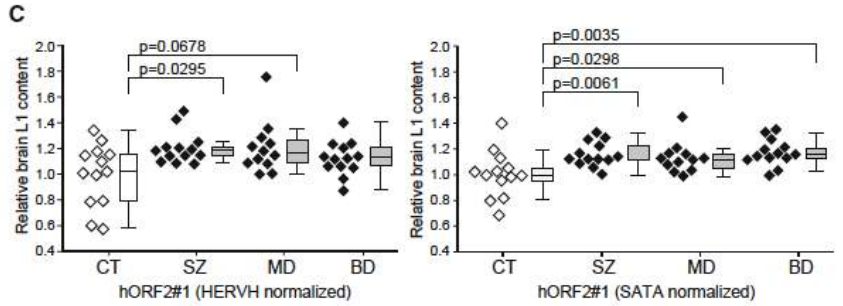
Miki Bundo,[1,2] Manabu Toyoshima,[3] Yohei Okada,[4] Wado Akamatsu,[4] Junko Ueda,[2] Taeko Nemoto-Miyauchi,[2] Fumiko Sunaga,[1] Michihiro Toritsuka,[5] Daisuke Ikawa,[5] Akiyoshi Kakita,[6] Motoichiro Kato,[7] Kiyoto Kasai,[8] Toshifumi Kishimoto,[5] Hiroyuki Nawa,[9] Hideyuki Okano,[4] Takeo Yoshikawa,[3] Tadafumi Kato,[2,*] and Kazuya Iwamoto[1,10,*]

- Increased L1 copy number in neurons from prefrontal cortex of patients and in induced pluripotent stem (iPS) cell-derived neurons containing 22q11 deletions.
- Whole-genome sequencing revealed brain-specific L1 insertion in patients localized preferentially to synapse- and schizophrenia-related genes.
- Test perinatal environmental risk factors for schizophrenia in animal models :
- Increased L1 copy number after immune activation by poly-I:C or epidermal growth factor.
- Hyperactive retrotransposition of L1 in neurons triggered by environmental and/or genetic risk factors
- **Contributes to susceptibility/pathophysiology of schizophrenia**?
- **Or just a consequence of factors implicated in the disease?**



B

set	diagnosis	n	gender (F:M)	age	onset (yrs)	suicide	side (R:L)	pH	PMI (hrs)
I	CT	13	5:8	48.2±10.4	-	0	5:8	6.2±0.2	23.6±10.7
I	SZ	13	5:8	44.4±12.9	24.2±8.1	3	6:7	6.2±0.3	34.7±14.6
I	MD	12	5:7	45.2±10.0	33.5±11.8	6	3:9	6.1±0.2	27.8±10.5
I	BD	13	5:8	41.5±11.2	21.9±8.9	7	7:6	6.1±0.2	31.2±15.2
II	CT	34	8:26	44.5±7.5	-	0	18:16	6.6±0.3	29.5±13.0
II	SZ	35	9:26	42.6±8.5	21.3±6.1	7	18:17	6.5±0.2	31.4±15.3



- NB Links between viral exposure and risk of schizophrenia
- Inflammation that seems to precede development of psychotic symptoms may be due to elevated levels of TE RNA activating immune response? (pure speculation!)

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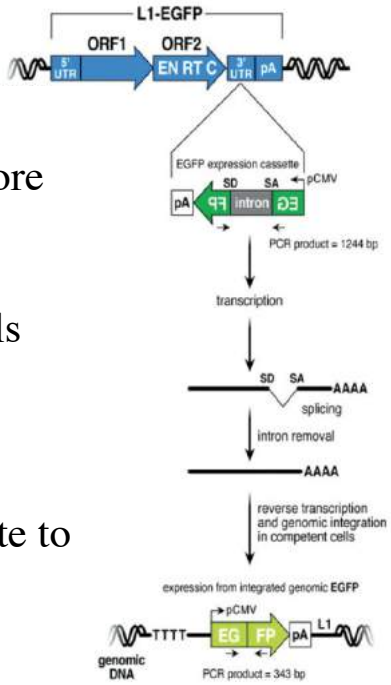
Exercise can influence L1 activity in the Brain

HIPPOCAMPUS 19:1002–1007 (2009)

Environmental Influence on L1 Retrotransposons in the Adult Hippocampus

Alysson R. Muotri,[1*] Chunmei Zhao,[2] Maria C.N. Marchetto,[2] and Fred H. Gage[2*]

- Neurons from mice that experience voluntary exercise are more likely to activate an EGFP reporter marker, representing L1 insertions in the brain, than sedentary animals.
- In the hippocampus, EGFP expression is mainly found in cells localized in the subgranular layer of the dentate gyrus.
- => neuronal progenitor cells may support *de novo* retrotransposition upon exposure to a new environment.
- => experience-dependent L1 retrotransposition may contribute to the physiological consequences of neuronal plasticity…



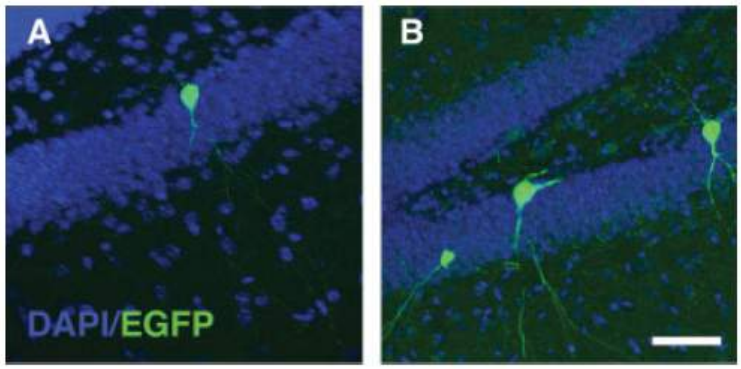
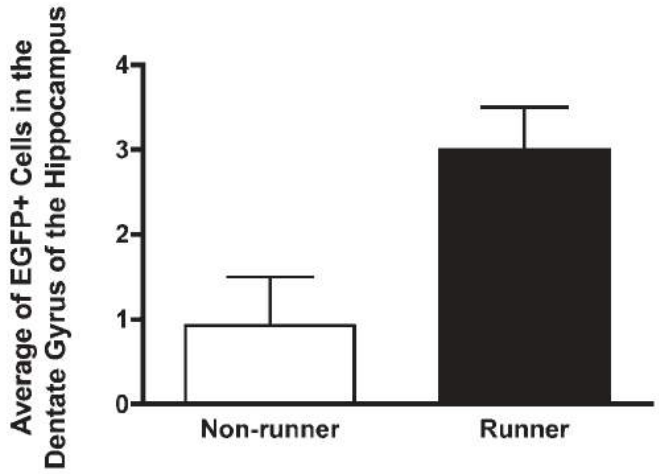


FIGURE 4. Somatic L1 retrotransposition in the adult hippocampus. EGFP expression in granular cells in the dentate gyrus of the hippocampus is increased in L1-EGFP transgenic mice previously exposed to voluntary exercise (B) when compared with sedentary animals (A). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



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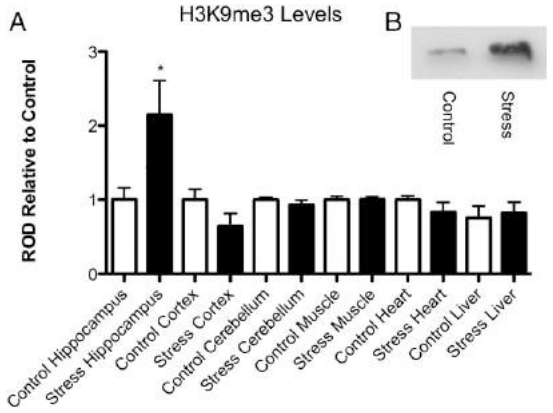
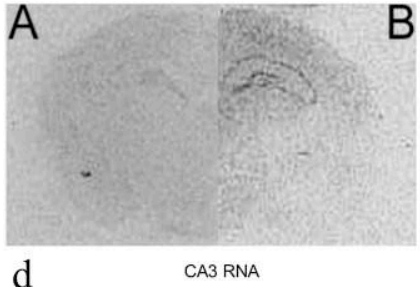
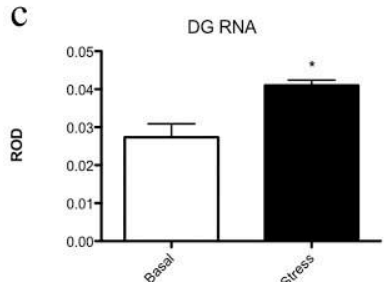
Stress and TE regulation

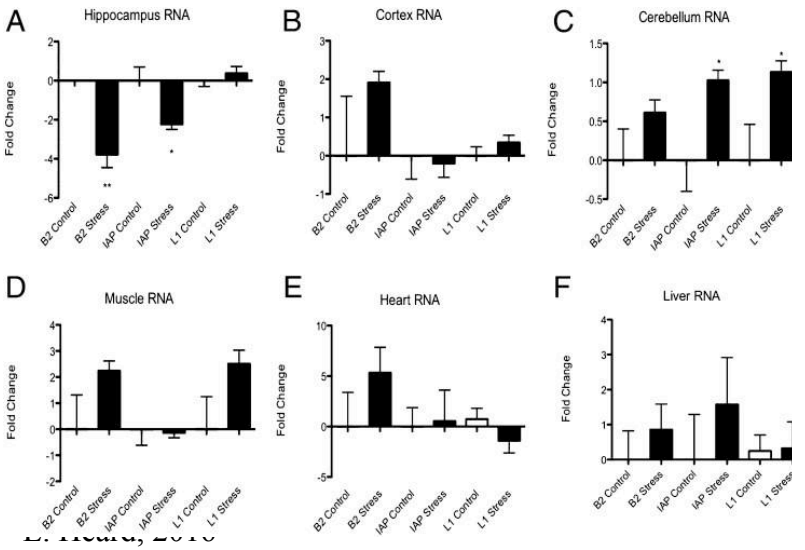
Acute stress and hippocampal histone H3 lysine 9 trimethylation, a retrotransposon silencing response

Richard G. Hunter[a,b,1], Gen Murakami[a], Scott Dewell[c], Ma'ayan Seligsohn[b], Miriam E. R. Baker[b], Nicole A. Datson[d], Bruce S. McEwen[b], and Donald W. Pfaff[a,1]









- Substantial, rapid (<2 h) and regionally selective increase in H3K9me3 within the rat hippocampus as a consequence of acute restraint stress.
- Affects most TEs looked at but does not seem to silence LINEs
- Persists for at least 24 h but habituate by 7d of repeated stress and was absent after 3 weeks (learning or adaptation?)
- Hippocampus is particularly susceptible to the effects of stress because of its high level of glucocorticoid receptors (GRs).

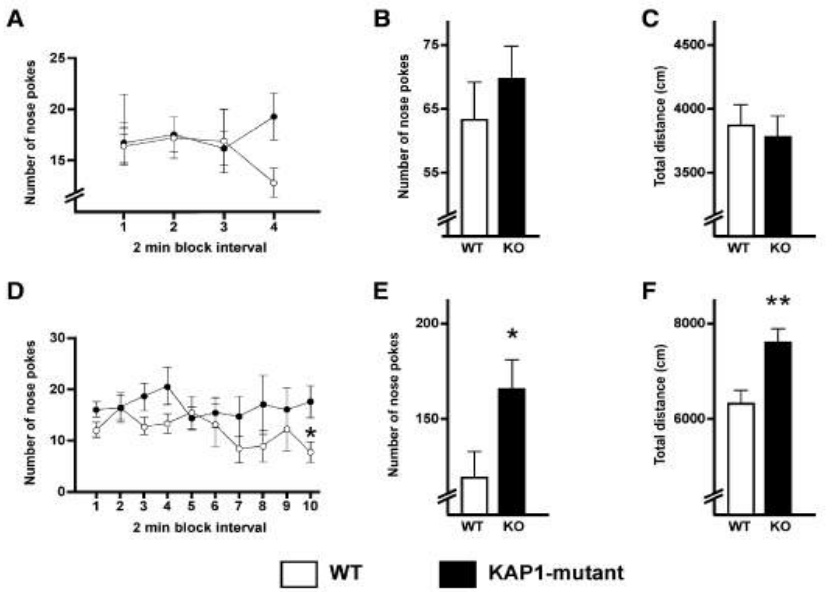
TEs and their KRAB-ZFPs can Control Gene Expression via Histone Modifications in Adult Tissues

KAP1-Mediated Epigenetic Repression in the Forebrain Modulates Behavioral Vulnerability to Stress

Johan Jakobsson,[1,2,5] Maria Isabel Cordero,[1] Reto Bisaz,[1] Anna C. Groner,[1,2] Volker Busskamp,[1,2] Jean-Charles Bensadoun,[1] Florence Cammas,[3] Régine Losson,[3] Isabelle M. Mansuy,[4] Carmen Sandi,[1,*] and Didier Trono[1,2,*]

- Deletion of Trim28 during brain development is lethal (Fasching et al, 2015)
- Deletion of Trim28 in postmitotic forebrain neurons results in complex behavioral changes (Jakobsson et al., 2008).
- Heterozygous germline deletion of Trim28 result in abnormal behavioral phenotypes (Whitelaw et al., 2010).
- In Hippocampus, depletion of KAP1/Trim28 leads to loss of H3K9me3 & deregulation of nearby genes (Jakobsson et al., 2008).

Heightened levels of anxiety-like and exploratory activity and stress-induced alterations in spatial learning & memory



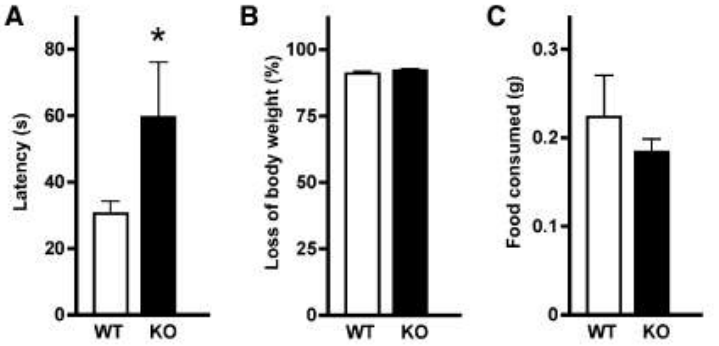


Figure 5. Conflict-Based Anxiety-like Behavior of KAP1 Mutant Mice in the Novelty Suppressed Feeding Test

Latency to approach the food pellet(s) (A), percentage of body weight lost after 24 hr food deprivation (B), amount of food consumed in the home cage during the 5 min post-NSF period (C). Results are the mean ± SEM. *$p < 0.05$ versus WT. WT n = 9, KO n = 9.

Stress and Epigenetic regulation of TEs

• McClintock first proposed that TE activation may be of adaptive use, under conditions of stress (McClintock B. The significance of responses of the genome to challenge. Science 1984; 226:792-801)

• Do epigenetic marks act as transient "stress memories"?
- they are sensitive to environmental stimuli
- stable enough to be maintained and impact future stress reactivity…
- eventually wear off?

• Past experience with stress influences subsequent genomic response to stress (and see changes in gene expression after multiple stressors).
• Epigenetic regulation of TEs in response to stress appears to be dependent on type of stress (heat shock leads to increased SINE/Alu RNA, acute restraint leads to decrease in SINE/Alu)
• Transciption Factor / KRAB-ZFP regulation of TEs and nearby genes after different stresses – exciting new field!
• Understanding the role of steroid receptor in regulation of TEs may help explain differences between sexes in prevalence of certain developmental disorders (see Lapp and Hunter, 2016).

Ageing and Epigenetic regulation of TEs

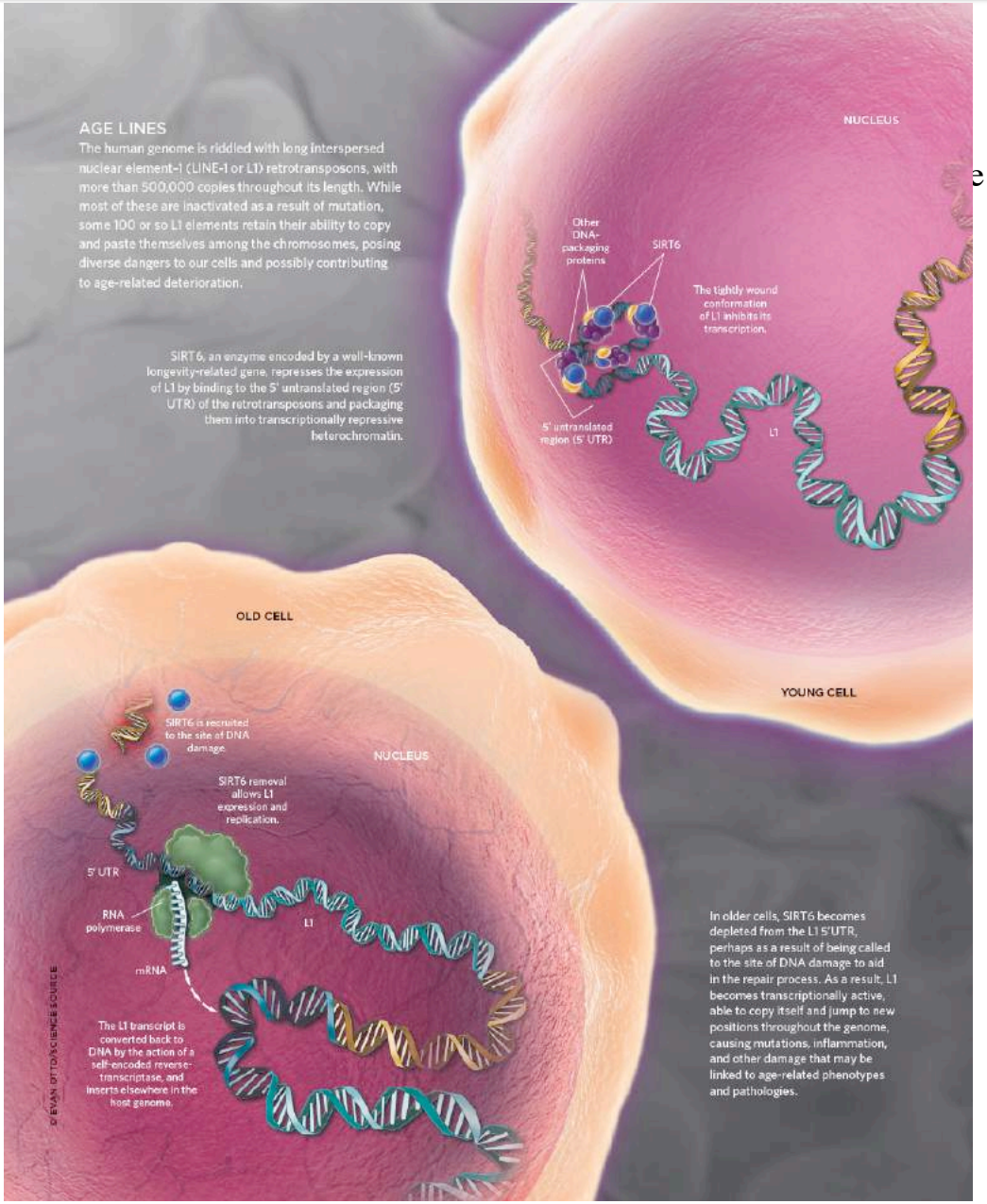
SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age

Michael Van Meter[1], Mehr Kashyap[1], Sarallah Rezazadeh[1], Anthony J. Geneva[1], Timothy D. Morello[1], Andrei Seluanov[1] & Vera Gorbunova[1]

- High L1 activity observed in aging tissues, particularly those affected by age-related patholgies such as cancer.
- Might L1 activity may *contribute* to the aging process?

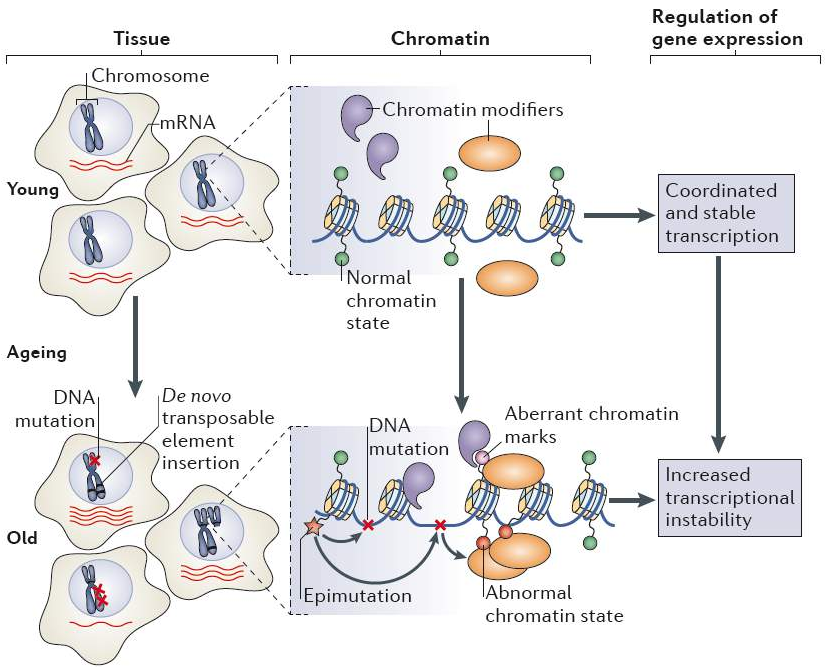
Longevity regulating protein, SIRT6, is a powerful repressor of L1 activity.
SIRT6 binds to the 5'UTR of L1, where it mono-ADP ribosylates TRIM28/KAP1, and facilitates interaction with heterochromatin factor, HP1a,
⇒ Reinforcing transcriptional repression.

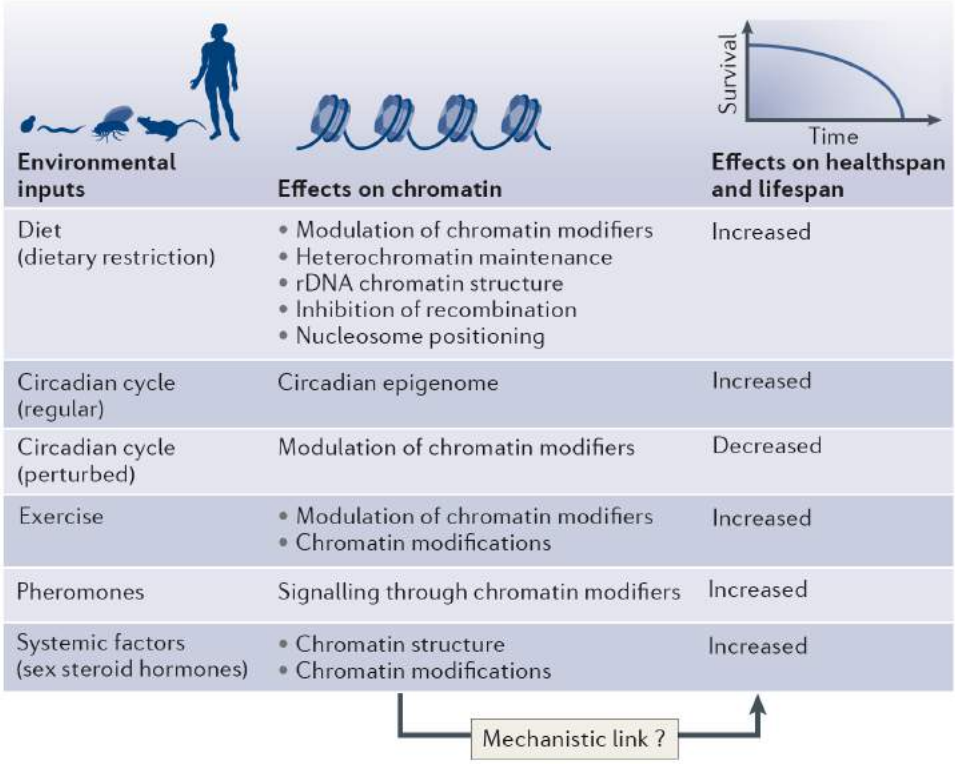
During the course of ageing, and also in response to DNA damage, SIRT6 is **depleted from L1 loci**, allowing the activation of previously silenced TEs.



E. Heard, February 6th, 2017

How do Epigenetic Changes Arise during Ageing?



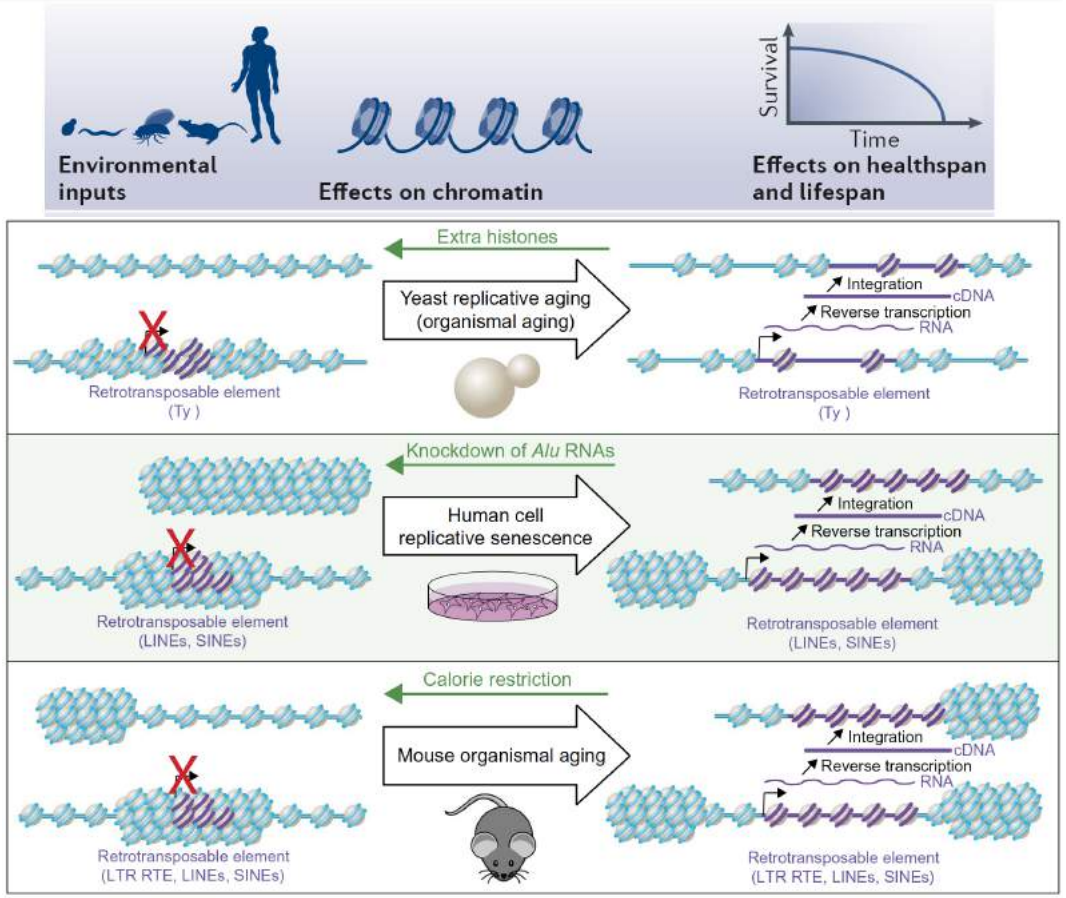


Benayoun et al, 2015

How do Epigenetic Changes Arise during Ageing?

- Epigenetic changes occur with age
- Epigenetic changes may influence aging
- Senescence of fibroblasts and aging mouse tissues are marked by progressive epigenetic reorganization, depression of TEs, increased insertions at late-stage senescence.
- Increased mobilization of TEs in ageing fly brain linked to neural & cognitive decline
- Epigenetic changes during aging result in altered local accessibility to genetic material, leading to aberrant gene expression, reactivation of transposable elements, and genomic instability.
- Increased mobility of retrotransposons, observed in the genomes of aged cells and tissues from multiples species, provides evidence for a hypothesized model of aging:

Aging by transposition:
TEs and their transposases may be a driving force to cause structural dysregulation of the genome to manifest aging phenotypes.



Pal and Tyler, 2016

Sedivy et al Death by transposition–the enemy within?
Bioessays 35, 1035–1043 (2013).
De Cecco et al Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. Aging 5:867–83 (2013)
Van Meter et al. SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age.
Nat Commun. 5:5011(2014)

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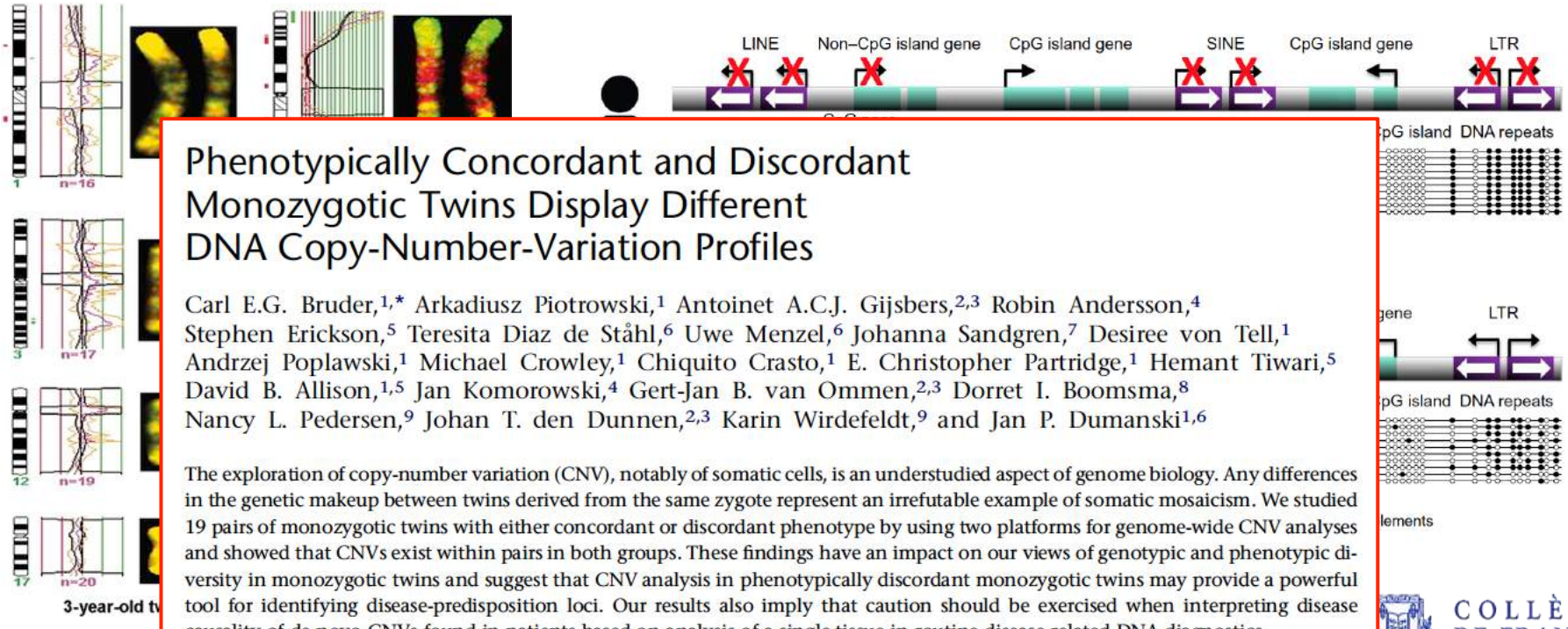
Ageing and Epigenetic changes influence TE and Gene expression

Just how similar are two supposedly genetically identical individuals as they age…



Phenotypically Concordant and Discordant Monozygotic Twins Display Different DNA Copy-Number-Variation Profiles

Carl E.G. Bruder,[1,*] Arkadiusz Piotrowski,[1] Antoinet A.C.J. Gijsbers,[2,3] Robin Andersson,[4] Stephen Erickson,[5] Teresita Diaz de Ståhl,[6] Uwe Menzel,[6] Johanna Sandgren,[7] Desiree von Tell,[1] Andrzej Poplawski,[1] Michael Crowley,[1] Chiquito Crasto,[1] E. Christopher Partridge,[1] Hemant Tiwari,[5] David B. Allison,[1,5] Jan Komorowski,[4] Gert-Jan B. van Ommen,[2,3] Dorret I. Boomsma,[8] Nancy L. Pedersen,[9] Johan T. den Dunnen,[2,3] Karin Wirdefeldt,[9] and Jan P. Dumanski[1,6]

The exploration of copy-number variation (CNV), notably of somatic cells, is an understudied aspect of genome biology. Any differences in the genetic makeup between twins derived from the same zygote represent an irrefutable example of somatic mosaicism. We studied 19 pairs of monozygotic twins with either concordant or discordant phenotype by using two platforms for genome-wide CNV analyses and showed that CNVs exist within pairs in both groups. These findings have an impact on our views of genotypic and phenotypic diversity in monozygotic twins and suggest that CNV analysis in phenotypically discordant monozygotic twins may provide a powerful tool for identifying disease-predisposition loci. Our results also imply that caution should be exercised when interpreting disease causality of de novo CNVs found in patients based on analysis of a single tissue in routine disease-related DNA diagnostics.

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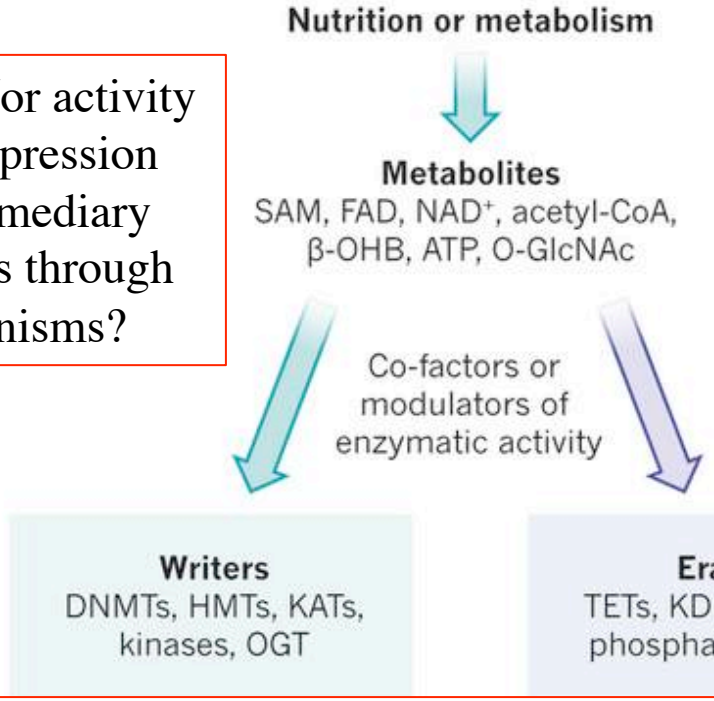
How do Epigenetic Changes Arise: Metabolic Stress

Metabolic stress and chromatin changes

Are TE mobility and/or activity and nearby gene expression influenced by intermediary metabolism products through epigenetic mechanisms?



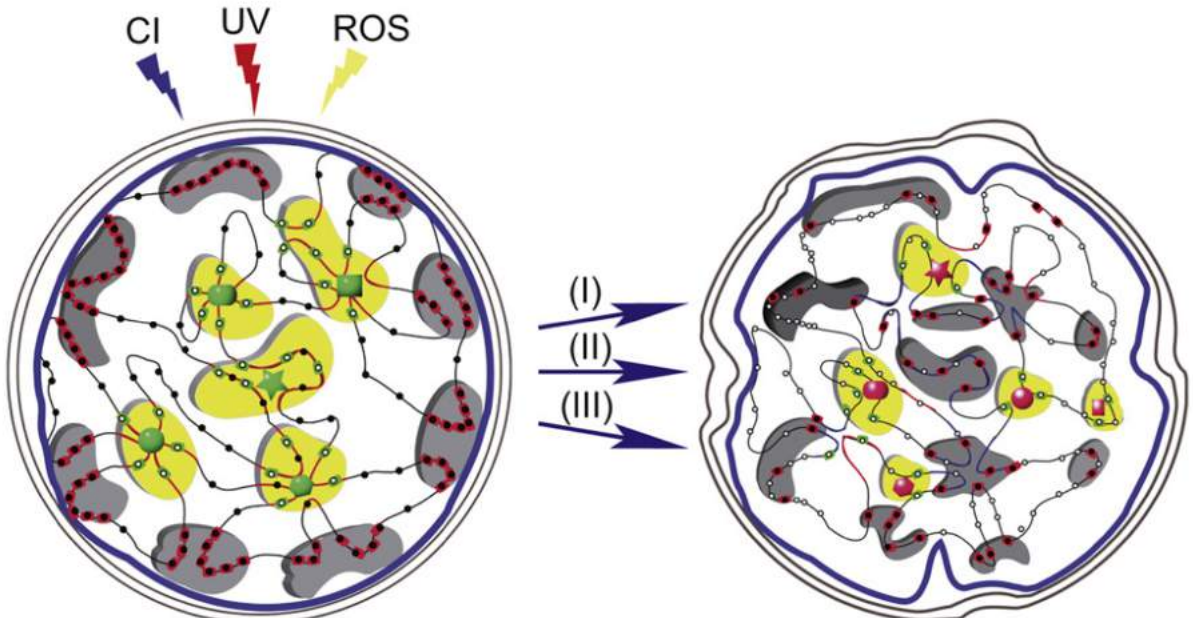
- Cellular concentrations of metabolites can fluctuate as a function of a cell's metabolic state
- => **the activity of chromatin regulators** may change as a function of **metabolic status** and so transduce a homeostatic transcriptional response?

Genetically well defined, *in vivo* models that can be used to capture epigenetic changes, & TE activity in response to temporarily restricted exposure to 'epigenetically toxic' metabolites (eg glucose!) are needed!

Kaelin, W. G. & McKnight, S. L. Influence of metabolism on epigenetics and disease. *Cell* **153,** 56–69 (2013).
Lu, C. & Thompson, C. B. Metabolic regulation of epigenetics. *Cell Metab*. **16,** 9–17 (2012).
Wellen & Thompson. A two-way street: reciprocal regulation of metabolism and signalling. *Nature Rev. Mol. Cell Biol*. **13,** 270–276 (2012).
Katada, S., Imhof, A. & Sassone-Corsi, P. Connecting threads: epigenetics and metabolism. *Cell* **148,** 24–28 (2012).
Teperino, R.et al Histone methyl transferases and demethylases; can they link metabolism and transcription? *Cell Metab*. **12,** 321–327 (2010)

How do Epigenetic Changes Arise: Oxidative Stress

Elevated levels of reactive oxygen species (ROS) arising from alterations in cellular metabolism and inflammatory responses constitute a key risk state for increased cancer susceptibility(Federico et al., 2007).
The major forms of oxidative DNA damage are nonbulky lesions such as 8-oxo-2'deoxyguanosine (8-oxo-dG) and thymine glycol that are repaired predominantly by base excision repair (BER) (Reardon et al., 1997).



Oxidative damage induces formation and relocalization of a silencing complex that may explain cancer-specific aberrant DNA methylation and transcriptional silencing

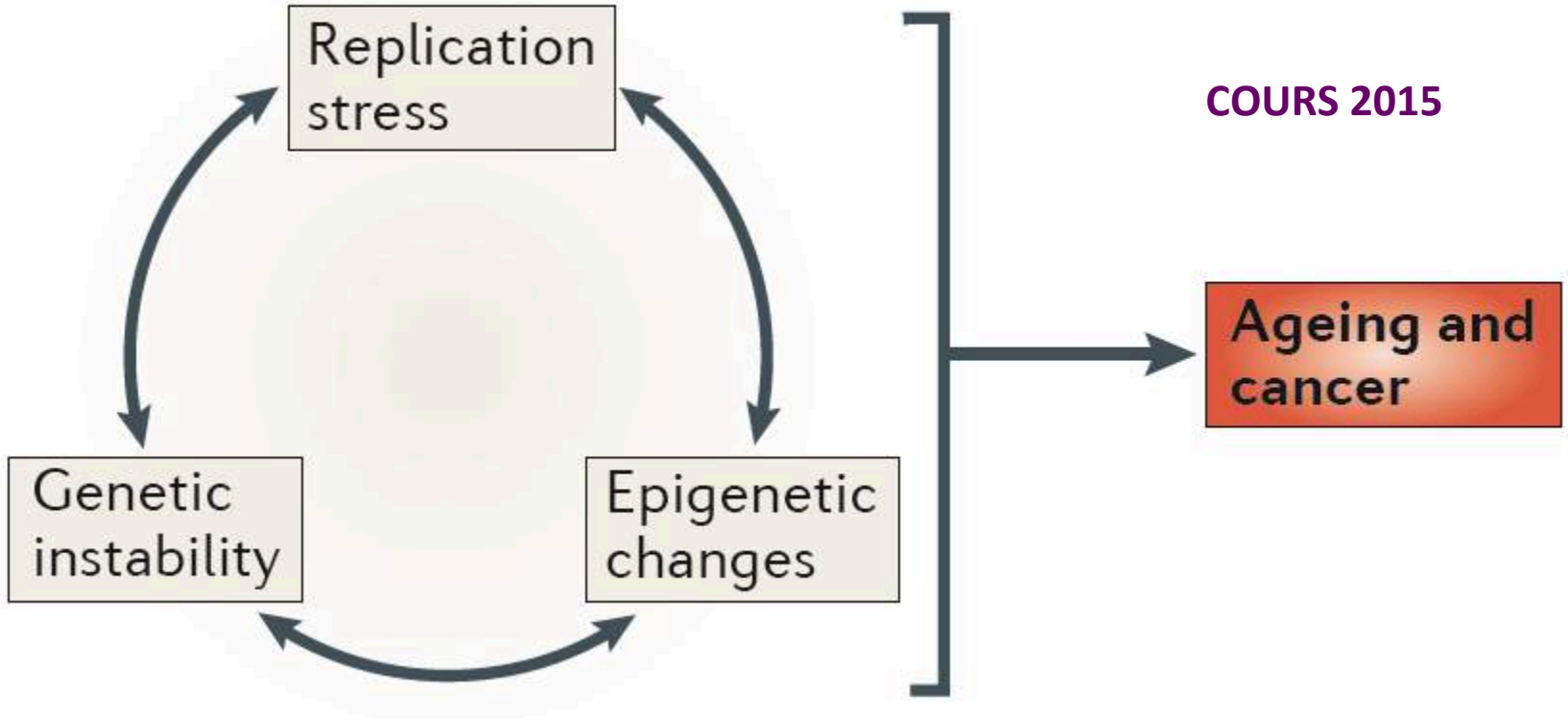
A potential role for increased levels of cellular ROS that accompany cancer risk states such as inflammation, in the formation of cancer-specific aberrant patterns of DNA methylation and transcriptional silencing?

When cells are exposed to chronic oxidative damage that is present during all phases of tumorigenesis, see induced shifts in chromosome localization -> may be associated with losses of DNA methylation observed in cancer cells. (O'Hagan et al, 2012, Cancer Cell)

Replication stress: loss of chromatin memory

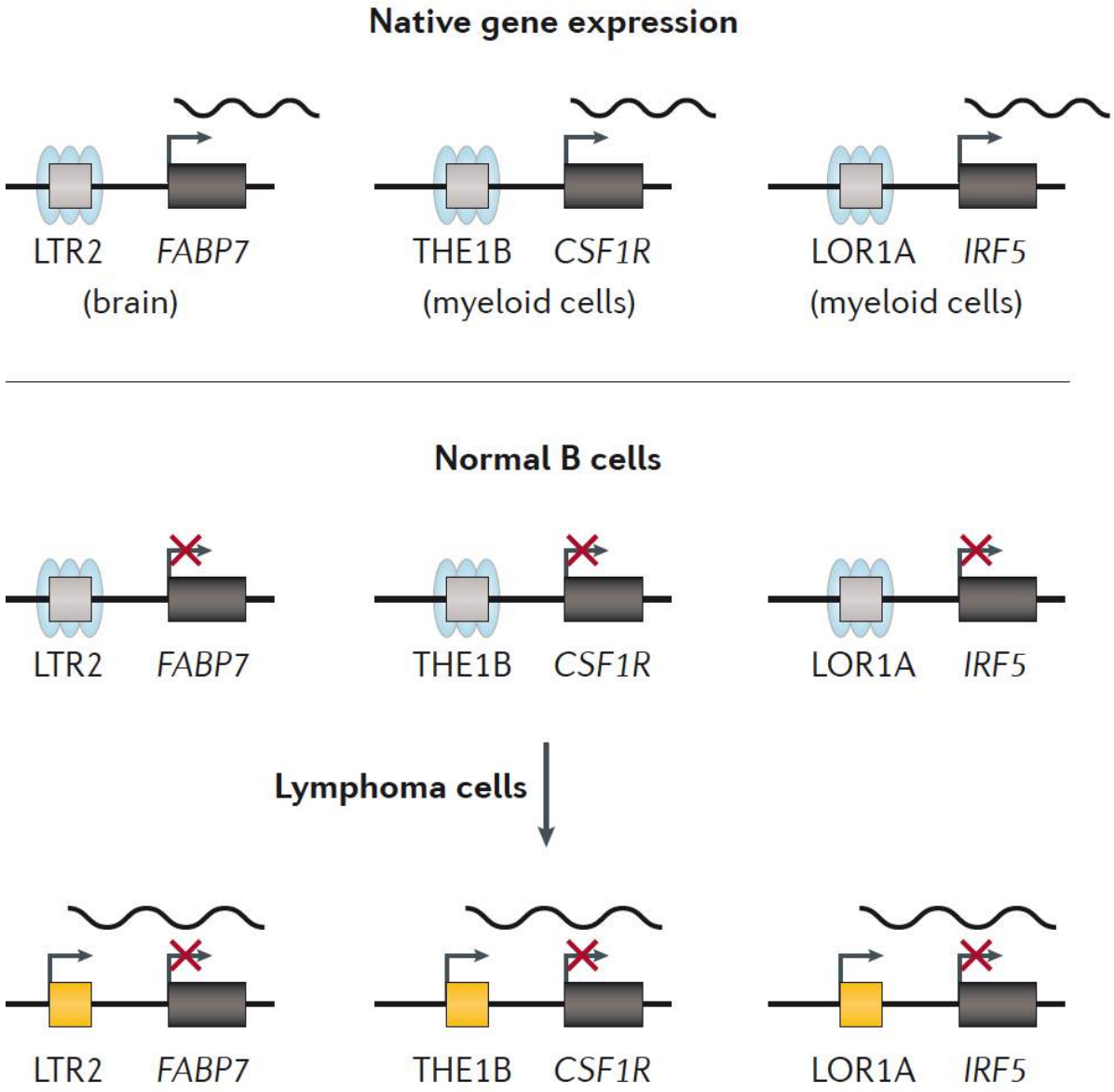
Replication stress can lead to both DNA mutations
and epigenetic changes (chromatin memory loss) that can impact on:
gene expression, repeat element activity, centromere function…
leading to further genetic and epigenetic aberrations

Oncogenic activity can trigger replication stress, including unscheduled initiation, fork stalling and collapse. This can result in epigenetic aberrations in cancer…



COURS 2015

TEs can be Aberrantly Reactivated to Promote Disease States



TEs as Genetic and Epigenetic Modifiers of Cancer

- First report in the late 1980s L1 retroelement insertion into the human proto-oncogene c-*myc* was found in human breast carcinoma cell
- First example of possible "**Onco-exaptation**"

Examples of TE insertion and TE-mediated chromosomal rearrangements associated with cancer.

Locus and/or gene	Associated cancer	TE	Distribution
Insertion			
APC, adenomatous polyposis coli gene	Desmoids tumors	*Alu*	Germline
APC	Colon cancer	L1	Germline
APC		L1	Somatic
BRCA1, breast cancer 1 gene	Breast/ovarian cancer	*Alu*	Germline
BRCA2, breast cancer 2 gene	Breast/ovarian cancer	*Alu*	Germline
MYC, c-myc proto-oncogene	Breast carcinoma	L1	Somatic
NF1, neurofibromatosis 1 gene	Neurofibroma	*Alu*	Germline
Chromosomal deletions			
VHL, von Hippel Lindau gene	von Hippel Lindau disease	*Alu*	Germline
BRCA1	Breast/ovarian cancers	*Alu*	Germline
BRCA2	Breast/ovarian cancers	*Alu*	Germline
CDH1, cadherin 1 gene	Hereditary diffuse gastric cancer	*Alu*	Germline
CAD, caspase activated DNase gene	Hepatoma	*Alu*	Somatic
Chromosomal duplication			
MLL1, myeloid/lymphoid mixed lineage leukemia gene	Acute myeloid leukemia	*Alu*	Somatic
MYB, myb transcription factor gene	T-acute lymphoblastic lymphoma	*Alu*	Somatic
BRCA1	Breast/ovarian cancers	*Alu*	Germline
Chromosomal translocation			
EWSR1-ETV, t(5q23q31)(18q12)	Ewing sarcoma	*Alu*	Somatic
BCR-ABL, t(9;22)(q34;q11)	Chronic myeloid leukemia	*Alu*	Somatic

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2016-2017 :
"Épigénétique et ADN égoïste"

Mercredi 8 mars, 2017, 16h00

Cours V

Contribution des éléments transposables et de leur contrôle épigénétique à l'évolution

Contributions of transposable elements and their epigenetic control in evolution

Seminaire (17h30): Prof. Rob Martienssen (CSH lab, USA)
"*Germline reprogramming and epigenetic inheritance: how to avoid Bad Karma*"